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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis, cytotoxicity, and molecular modeling study of 2,4,6-trisubstituted pyrimidines with anthranilate ester moiety

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Abstract

A series of 2,4,6-trisubstituted pyrimidines with antharanilic acid ester moiety have been designed and synthesized by employing a one-pot multicomponent approach from methyl 5-(ethynyl)anthranilate, aroyl or cinnamoyl chlorides and various amidines. All the compounds were evaluated for their cytotoxic activity with respect to model cancer cell lines (CEM-13, U-937, MDA-MB-231, BT-474, DU-145) using conventional MTT assays. Structure-activity relationship analysis revealed that 4,6-diarylpyrimidines **13**–**17**, substituted with a pyridine or a pyrimidine ring in the 2 position displayed an increasing of activity compared to 2-methyl or 2-phenyl substituted pyrimidines. The 2-amino substututed compound **9** showed selectivity on the human monocyte-like cells U-937. Trisubstituted pyrimidines **18**–**21**, containing a (E)-styryl substituent in the 6 position of the pyrimidine core, demonstrated an increasing of activity against the breast cancer cell lines MDA-MB-231, BT-474, and also against the human prostate cell lines DU-145. Compounds **18** and **20** shown the best potency towards the cancer cell lines MDA-MB-231; theirs activity was comparable with the activity of Doxorubicin on this cell lines. These compounds were confirmed to be cyclin-dependent kinase 9 (CDK9) inhibitors through in silico molecular modeling studies for the mode of action. The newly synthesized compounds may serve as lead molecules for the future research regarding the identification of new anticancer agents in the pyrimidine series.

Keywords Pyrimidines · Alkynes · Multicomponent reactions · Cytotoxicity · Molecular docking · CDK inhibitors

Introduction

Pyrimidines represent one of the most important heterocycles found in many kinds of functional compounds, such as natural products (Meridianins, Variolin B), drugs (preparation Sulfadiazine, Imatinib, Dasatinib), pharmaceutically important molecules (Rosuvastatin), supramolecules and photophysical materials (Fig. 1) (Capdeville et al. 2002, Quintas-Cardama et al. 2007, Walker et al. 2009).

Much attention has been directed toward pyrimidines with biological activities, including antibacterial (Keche et al. 2012, Mandi 2017), antileishmanial (Sunduru et al. 2009), anti-viral (Rawal et al. 2007, Liu et al. 2014, Huo et al. 2018), anti-inflammatory (Martin et al. 2006, Chhabria et al. 2007, Undare et al. 2016), antiproliferative and antitumor (Sherif et al. 2009, Burger et al. 2011, Zhu et al. 2012, Chan et al. 2015). Some pyrimidine derivatives were identified as attractive agent for treatment of neurological disorders (Shipe et al. 2015, Rehman et al. 2017, Kumar et al. 2018) and metabolic abnormalities (Ryu et al. 2016).

The activities of pyrimidines has been linked to the diversity of substituents that can be introduced, especially on the C-2, C-4, and C-6 positions. So, 5-ethoxycarbonyl-6-isopropylamino-4-(aryl)aminopyrimidines (Chhabria et al. 2007) and 4-hydroxy-2-methyl-6-phenylpyrimidine-5-carbonitrile derivatives (Undare et al. 2016) were characterized as anti-inflammatory agents. In a series of 4-(3-(trifluoromethyl)phenylamino-6-arylpyrimidines bearing additional urea, thiourea, and sulfonamide moieties in the 4 position on the aryl ring compounds with potent anti-

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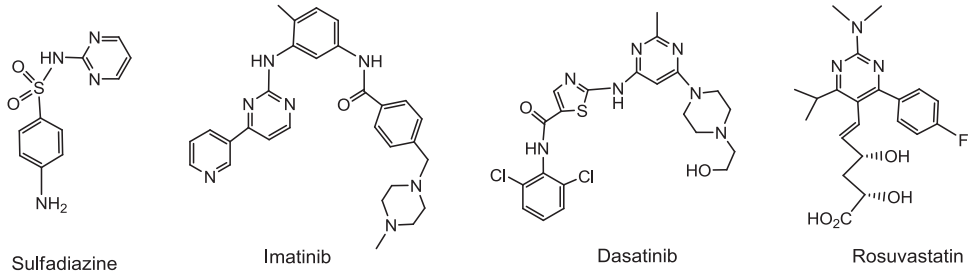


Fig. 1 Representative pyrimidine compounds

inflammatory and promising antimicrobial activity against pathogenic bacteria and fungi were found (Keche et al. 2012). Pyrimidines containing a thiazolidin-4-one (Rawal et al. 2007) or 2-(4-cyanoaryl)amino substituent (Liu et al. 2014) in the 2 position were studied as anti-HIV-1 agents. 6-(Pyridin-3-yl)-2,4-bismorpholino pyrimidine (NVP-BKM120) (Burger et al. 2011) and 6-hydrazinyl-2,4-bismorpholino pyrimidine (Zhu et al. 2012) exhibited moderate to excellent antiproliferative activity against cancer cell lines. In the group of 2,4-diamino-5-aroylpyrimidines the cytotoxicity of the compounds also dependent on the substitution in the ring. So, (N-methanesulfonylpiperidin-4-yl)amino- in the C-2 position and 2-methoxybenzoyl substituent in the C-5 position are the most preferred group (Chu et al. 2006). Studying of a series of 4-aryl-6-(piperidin-1-yl)pyrimidines bearing a sulfonamide group in the piperidine moiety, against human tumor cell lines revealed that the grown inhibition activities has been enhanced by the introduction of a bulky substituent on the aromatic ring (Lefebvre et al. 2017). Substitution on the C-4 position of 2-anilinopyrimidines bearing a strobilurin moiety was found to produce a group of antitumor agents which were successfully used for the treatment of chronic myeloid leukemia (Chai et al. 2013). Furthermore, more studies based on the pyrimidine scaffold toward antitumor activity involving the analysis of different targets (e.g., CDKs, ALK, PDK1, EGFR, II PDGFRα kinase) (Wang et al. 2004, Chu et al. 2006, Lee et al. 2014, Chan et al. 2015, Wucherer-Plietker et al. 2016, Singh et al. 2017, Song et al. 2017, Czudor et al. 2018, Wang et al. 2018a, b) have been carried out. So, the pyrimidine scaffold was found to be a remarkable building block and around it a variety of novel heterocycles with excellent pharmaceutical profile can be designed.

Herein we describe an approach to pyrimidines containing a methyl anthranilate moiety in the 4 position. Anthranilic acid derivatives are widely distributed in numerous natural and synthetic products and demonstrated a variety of biological properties suggestive of possible clinical applications as anti-thrombotic compounds devoid of risk of bleeding and also as agents capable of preventing or treating inflammation, multiple sclerosis and tumor (Merk et al. 2015, Placencio et al. 2015). There was therefore value in a targeted preparation of pyrimidines with anthranilic acids substituent.

Traditionally, pyrimidine structures are constructed by the condensation of amidines with 1,3-dicarbonyl compound, α,β-unsaturated ketones, or allylic aryl compounds (Hill and Movassaghi 2008, Odom and McDaniel 2015, Mahfoudh et al. 2017). In recent years, numerous attempts have been made toward the synthesis of pyrimidines from more available nitrogen sources, under milder reaction conditions, and more facile operational procedures including multicomponent reaction strategy (Karpov and Müller 2003a, b, Karpov et al. 2005, D'Souza and Müller 2008, Willy and Müller 2008, Guo et al. 2017, Schmidt et al. 2017). This prompted us to design an approach to anthranilic acid ester substituted pyrimidines through the modified Sonogashira reaction of 5-(ethynyl)methylanthranilate **1** (Osadchii et al. 2007) with acid chlorides and the reaction of obtained alkynyl ketones with amidines in a one-pot manner. We also report the cytotoxicity of the newly synthesized compounds on human cancer cell lines and molecular docking studies towards CDK-6 and CDK-9 kinases.

Materials and methods

IR spectra were recorded on a Bruker Vector 22 FTIR spectrometer in KBr pellets. ^{1}H and ^{13}C NMR spectra were acquired on Bruker AV-400 (400.13 (^{1}H), 100.76 (^{13}C) MHz) or DRX-500 (500.13 (^{1}H), 125.76 (^{13}C) MHz) spectrometers (Bruker Company, Germany) in $CDCl_3$ or DMSO-d_6 as solvents, using TMS as internal standard. Chemical shift was expressed in ppm (δ). NMR assignments were supported using COSY, HMBC, and HMQC spectra where appropriate. The mass spectra were recorded on a ThermoScientific DFS high-resolution mass spectrometer (evaporator temperature 200–250 °C, EI ionization at 70 eV). Melting points were determined using Stuart SMP30 melting point apparatus (Bibby Scientific, Staffordshire, UK).

The reaction progress and the purity of the obtained compounds were monitored by TLC on Silufol UV-254 plates (Kavalier, Czech Republic, $CHCl_3$-EtOH, 100:1; detection under UV light or by spraying the plates with 10%

water solution of H_2SO_4 followed by heating at 100 °C). Preparative column chromatography was carried out on 60H silica gel (0.063–0.200 mm, Merck KGaA, Darmstadt, Germany) with the indicated solvent systems. Oxygen or water sensitive reactions were performed under the argon atmosphere. The starting materials: methyl 2-(*N*-acetylamino)-5-ethynylbenzoate **1** (Osadchii et al. 2007), 3-pyridinecarboximidamide **33** (Medwid et al. 1990) and 2-amidinopyrimidine hydrochloride **34** (Lepri et al. 2016), were synthesized according to previously published procedures. Other reagents were purchased from commercial sources and were used without further purification. Solvents (THF, CH_2Cl_2, benzene, $CHCl_3$, MeCN, MeOH) and Et_3N were purified by standard methods and distilled in a stream of argon just before use. Purity of all compounds was checked by TLC.

General procedure for the synthesis of pyrimidines (2–10, 18–21). (Conditions *c*)

A mixture of alkynyl ketones **22–25** (1 mmol), amidine hydrochlorides (**30**, **31**) (1.5 mmol) or guanidine carbonate **32** (1.5 mmol), and Na_2CO_3 (3 mmol) was reflux in MeCN (30 mL) about 6 h (TLC). After keeping at r.t. for 15 h a precipitate was formed. The precipitate was filtered of, dried and washed with $CHCl_3$. The combined solution was concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel. Eluting with chloroform and crystallization from MeCN gave compound **2–10** (yield 56–82%). Compounds **18–21** were prepared by the reaction of en-ynones **38–40** with amidine hydrochlorides **30**, **31**. The procedures (*a*) and (*b*) for synthesis of compounds **22–25** and **38–40** are described in Suppl. Part.

General procedure for the synthesis of pyrimidines (11–17) (Conditions *d*)

To a solution of compound **1** (434 mg, 2.0 mmol) in CH_2Cl_2 (25 mL) were added carboxylic acid chloride (2.2 mmol), palladium clloride (II) (0.03 mmol), 1-(Ad)$_2$PBn•HBr (0.06 mmol) at r.t. under argon. The mixture was reflux about 2 h (TLC). The solvent was removed under reduced pressure, then solution of amidine (2.8 mmol) and Na_2CO_3 (6 mmol) in MeCN (30 mL) (or MeOH in the reaction with amidines **33**, **34**) was added to the residue. The reaction mixture was reflux for 6 h. After cooling the precipitate was filtered of, dried and washed with $CHCl_3$. The filtrate was concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel (eluting with chloroform) afforded target compounds **11–17**, which were additional purified by crystallization from CH_3CN.

Methyl 2-acetylamino-5-(2-methyl-6-phenylpyrimidin-4-yl) benzoate (2)

Colorless needles. Yield 70%; m.p. 165–166 °C; IR (KBr) ν_{max} 3265 (NH), 1703, 1691 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ = 11.22 (s, 1H, H-10), 8.86 (d, J = 8.9 Hz, 1H, H-3), 8.82 (d, J = 2.2 Hz, 1H, H-6), 8.27 (dd, J_1 = 8.9 Hz, J_2 = 2.2 Hz, 1H, H-4), 8.10 (m, 2H, H-2′,6′), 7.84 (s, 1H, H-5′′), 7.51 (m, 3H, H-3′,4′,5′), 3.98 (s, 3H, H-9), 2.84 (s, 3H, H-7′′), 2.26 (s, 3H, H-12); ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 169.11 (C-11), 168.47 (C-7), 168.36 (C-2′′), 164.86 (C-6′′), 162.89 (C-4′′), 143.20 (C-2), 137.19 (C-1′), 132.97 (C-4), 131.12 (C-5), 130.56 (C-6), 129.63 (C-4′), 128.79 (C-3′,5′), 127.09 (C-2′,6′), 120.38 (C-3), 114.87 (C-1), 109.23 (C-5′′), 52.43 (C-9), 26.31 (C-12), 25.46 (C-7′′); HRMS (ESI+) (*m/z*): calcd for $C_{21}H_{19}N_3O_3$ $[M+H]^+$: 361.1421; found: 361.1420.

Methyl 2-acetylamino-5-(2,6-diphenylpyrimidin-4-yl) benzoate (3)

Colorless needles. Yield 70%; m.p.: 221–223 °C; IR (KBr) ν_{max} 3263 (NH), 1703, 1691 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ = 11.19 (s, 1H, H-10), 8.87 (d, J = 8.9 Hz, 1H, H-3), 8.86 (d, J = 2.3 Hz, 1H, H-6), 8.70–8.64 (m, 2H, H-2′′′,6′′′), 8.40 (dd, J_1 = 8.9 Hz, J_2 = 2.3, 1H, H-4), 8.29–8.20 (m, 2H, H-2′,6′), 7.89 (s, 1H, H-5′′), 7.59–7.47 (m, 6H, H-3′,4′,5′,3′′′, 4′′′,5′′′), 3.98 (s, 3H, H-9), 2.24 (s, 3H, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 169.05 (C-11), 168.41 (C-7), 164.69 (C-2′′), 164.39 (C-6′′), 162.92 (C-4′′), 143.40 (C-2), 137.92 (C-1′′′), 137.31 (C-1′), 133.11 (C-4), 131.24 (C-5), 130.72 (C-4′′′), 130.58 (C-4′), 129.58 (C-6), 128.79 (C(-2′′′,6′′′), 128.37 (C-3′′′,5′′′), 128.33 (C-3′,5′), 127.18 (C-2′,6′), 120.42 (C-3), 114.87 (C-1), 109.40 (C-5′′), 52.48 (C-9), 25.44 (C-12); HRMS (ESI+) (*m/z*): calcd for $C_{26}H_{21}N_3O_3$ $[M+H]^+$: 423.1577; found: 423.1570.

Methyl 2-acetylamino-5-(2-amino-6-phenylpyrimidin-4-yl) benzoate (4)

Colorless needles. Yield 61%; m.p. 230–232 °C; IR (KBr) ν_{max} 3212 (N-H), 1703, 1688 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ = 10.70 (s, 1H, H-10), 8.73 (d, J = 1.9 Hz, 1H, H-6), 8.41 (dd, J_1 = 1.9 Hz, J_2 = 8.8 Hz, 1H, H-4), 8.37 (d, J = 8.8 Hz, 1H, H-3), 8.18 (m, 2H, H-2′,6′), 7.68 (s, 1H, H-5′′), 7.49 (m, 3H, H-3′,4′,5′), 6.80 (s, 2H, H-7′′), 3.88 (s, 3H, H-9), 2.13 (s, 3H, H-12); ^{13}C NMR (DMSO-d_6,125 MHz): δ = 169.03 (C-11), 167.83 (C-7), 165.31 (C-2′′), 164.35 (C-6′′), 163.53 (C-4′′), 141.61 (C-2), 137.54 (C-1′), 132.66 (C-4), 132.00 (C-5), 130.84 (C-6), 129.25 (C-4′), 128.94 (C-3′,5′), 127.32 (C-2′,6′), 121.04 (C-3), 117.90 (C-1), 101.90 (C-5′′), 53.06 (C-9), 25.28

(C-12); HRMS (ESI+) (*m/z*): calcd for $C_{20}H_{18}N_3O_4$ $[M+H]^+$: 362.1373; found: 362.1370.

Methyl 2-acetylamino-5-(6-(4-methoxyphenyl)-2-methylpyrimidin-4-yl)benzoate (5)

Colorless needles. Yield 82%; m.p. 196–198 °C; IR (KBr) ν_{max} 3261 (NH), 1707, 1685 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 11.21$ (s, 1H, H-10), 8.86 (d, $J =$ 8.9 Hz, 1H, H-3), 8.81 (d, $J = 2.1$ Hz, 1H, H-6), 8.25 (dd, $J_1 = 8.9$ Hz, $J_2 = 2.1$ Hz, 1H, H-4), 8.10 (d, $J = 8.8$ Hz, 2H, H-2′,6′), 7.78 (s, 1H, H-5′′), 7.02 (d, $J = 8.8$ Hz, 2H, H-3′,5′), 3.99 (s, 3H, H-8′), 3.87 (s, 3H, H-9), 2.82 (s, 3H, H-7′′), 2.26 (s, 3H, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C: 169.06 (C-11), 168.43 (C-7), 168.34 (C-2′′), 164.24 (C-6′′), 162.64 (C-4′′), 161.73 (C-4′), 143.11 (C-2), 132.95 (C-4), 131.38 (C-5), 129.61 (C-6), 129.58 (C-1′), 128.67 (C-2′,6′), 120.41 (C-3), 114.90 (C-1), 114.13 (C-3′,5′), 108.28 (C-5′′), 55.29 (C-8′), 52.41 (C-9), 26.34 (C-12), 25.46 (C-7′′); HRMS (ESI+) (*m/z*): calcd for $C_{22}H_{21}N_3O_4$ $[M+H]^+$: 391.1527; found: 391.1531.

Methyl 2-acetylamino-5-(6-(4-methoxyphenyl)-2-phenylpyrimidin-4-yl)benzoate (6)

Colorless needles. Yield 56%; m.p. 207–208 °C; IR (KBr) ν_{max} 3267 (N-H), 1703, 1688 (C=O cm^{-1}); ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 11.23$ (s, 1H, H-10), 8.91 (d, $J =$ 2.0 Hz, 1H, H-6), 8.90 (d, $J = 9.0$ Hz, 1H, H-3), 8.68 (dd, $J_1 = 8.0$ Hz, $J_2 = 1.9$ Hz, 2H, H-2′′′,6′′′), 8.43 (dd, $J_1 = 9.0$ Hz, $J_2 = 2.2$ Hz, 1H, H-4), 8.26 (d, $J = 8.8$ Hz, 2H, H-2′,6′)), 7.88 (s, 1H, H-5′′), 7.53 (m, 3H, H-3′′′,4′′′, 5′′′), 7.06 (d, $J = 8.9$ Hz, 2H, H-3′,5′)), 4.01 (s, 3H, H-8′), 3.90 (s, 3H, H-9), 2.27 (s, 3H, H-12); ^{13}C-NMR ($CDCl_3$, 125 MHz): $\delta = 169.15$ (C-11), 168.49 (C-7), 164.24 (C-2′′), 164.16 (C-6′′), 162.71 (C-4′′), 161.85 (C-4`), 143.28 (C-2), 137.98 (C-1′′′), 133.15 (C-4), 131.46 (C-5), 130.53 (C-4′′′), 129.65 (C-1′), 129.60 (C-6), 128.70 (C-2′,6′), 128.33 (C-3′′′,5′′′), 128.30 (C-2′′′,6′′′), 120.40 (C-3), 114.83 (C-1), 114.13 (C-3′,5′), 108.64 (C-5′′), 55.35 (C-8′), 52.55 (C-9), 25.53 (C-12); HRMS (ESI+) (*m/z*): calcd for $C_{27}H_{23}N_3O_4$ $[M+H]^+$: 453.1683; found: 453.1680.

Methyl 2-acetylmino-5-(6-(3,4-dimethoxyphenyl)-2-methylpyrimidin-4-yl)benzoate (7)

Colorless needles. Yield 61%; m.p. 180–181 °C; IR (KBr) ν_{max}: 3274 (N-H), 1710, 1687 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 11.18$ (s, 1H, H-10), 8.74 (d, $J =$ 8.9 Hz, 1H, H-3), 8.73 (d, $J = 1.7$ Hz, 1H, H-6), 8.17(dd, $J_1 = 8.9$ Hz, $J_2 = 1.7$ Hz, 1H, H-4), 7.73 (s, 1H, H-5′′), 7.67 (d, $J = 1.2$ Hz, 1H, H-2′), 7.58 (dd, $J = 8.0$ Hz, $J_2 = 1.2$ Hz, 1H, H-6′), 6.92 (d, $J = 8.0$ Hz, 1H, H-5′), 3.93 (s, 3H, H-10′), 3.91 (s, 3H, H-8′), 3.87 (s, 3H, H-9), 2.76 (s, 3H, H-7′′)), 2.19 (s, 3H, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 169.10$ (C-11), 168.40 (C-7), 168.23 (C-2′′), 164.22 (C-6′′), 162.66 (C-4′′), 151.32 (C-4′), 149.22 (C-3′), 143.19 (C-2), 132.94 (C-4), 131.23 (C-5), 129.81 (C-1′), 129.67 (C-6), 120.35 (C-3), 120.19 (C-5′), 114.89(C-1), 110.83 (C-6′), 109.88 (C-2′), 108.49 (C-5′′), 55.97 (C-8′), 55.89 (C-10′), 52.46 (C-9), 26.32 (C-7′′), 25.48 (C-12); HRMS (ESI+): (*m/z*): calcd for $C_{23}H_{23}N_3O_5$ $[M+H]^+$: 421.1632; found: 421.1626.

Methyl 2-acetylamino-5-(6-(3,4-dimethoxyphenyl)-2-phenylpyrimidin-4-yl)benzoate (8)

Colorless solid. Yield 68%; m.p. 184–185 °C; IR (KBr) ν_{max} 3273 (N-H), 1705, 1688 (C=O) cm^{-1}; ^1H-NMR ($CDCl_3$, 400 MHz): $\delta = 11.24$ (s, 1H, H-10), 8.94 (d, $J =$ 2.0 Hz, 1H, H-6), 8.91 (d, $J = 8.9$ Hz, 1H, H-3), 8.67 (d, $J_1 = 8.8$ Гц, $J_2 = 1.7$ Hz, 2H, H-2′′′, 6′′′), 8.43 (dd, $J_1 = 8.9$ Hz, $J_2 = 2.0$ Hz, 1H, H-4), 7.94 (d, $J = 1.6$ Hz, 1H, H-2′), 7.90 (s, 1H, H-5′′), 7.81 (dd, $J_1 = 8.4$ Hz, $J_2 = 1.6$ Hz, 1H, H-6′), 7.53 (m, 3H, H-3′′′,4′′′,5′′′), 7.02 (d, $J = 8.4$ Hz, 1H, H-5′), 4.06 (s, 3H, H-10′), 4.02 (s, 3H, H-8′), 3.97 (s, 3H, H-9), 2.28 (s, 3H, H-12); ^{13}C-NMR ($CDCl_3$,100 MHz): $\delta =$ 169.14 (C-11), 168.45 (C-7), 164.15 (C-2′′), 164.13 (C-6′′), 162.63 (C-4′′), 151.42 (C-4′), 149.19 (C-3′), 143.29 (C-2), 137.93 (C-1′′′), 133.07 (C-4), 131.30 (C5), 130.56 (C-4′′′), 129.95 (C-1′), 129.60 (C-6), 128.35 (C-2′′′,6′′′), 128.27 (C-3′′′,5′′′), 120.34 (C-3), 120.23 (C-5′), 114.80 (C-1), 110.80 (C-6′), 109.94 (C-2′), 108.77 (C-5′′), 56.00 (C-8′), 55.93 (C-10′), 52.55 (C-9), 25.52 (C-12); HRMS (ESI+) (*m/z*): calcd for $C_{28}H_{25}N_3O_5$ $[M+H]^+$: 483.1789; found: 483.1784.

Methyl 2-acetylamino-5-(2-amino-6-(3,4-dimethoxyphenyl)-pyrimidin-4-yl)benzoate (9)

Colorless solid. Yield 78%; m.p. 232–234 °C. IR (KBr) ν_{max} 3463, 3354 (H-NH), 3268 (AcN-H), 1704, 1688 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 11.22$ (s, 1H, H-10), 8.85 (d, $J = 8.9$ Hz, 1H, H-3), 8.79 (d, $J = 2.1$ Hz, 1H, H-6), 8.20 (dd, $J_1 = 8.9$ Hz, $J_2 = 2.1$ Hz, 1H, H-4), 7.74 (d, $J = 2.0$ Hz, 1H, H-2′), 7.63 (dd, $J_1 = 8.5$ Hz, $J_2 =$ 2.0 Hz, 1H, H-6′), 7.39 (s, 1H, H-5′′), 6.96 (d, $J = 8.5$ Hz, 1H, H-5′), 5.54 (s, 2H, H-7′′), 4.01(s, 3H, H-10′), 3.98 (s, 3H, H-8′), 3.95 (s, 3H, H-9), 2.26 (s, 3H, H-12); ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta = 169.10$ (C-11), 168.40 (C-7), 168.23 (C-2′′), 164.22 (C-6′′), 162.66 (C-4′′), 151.32 (C-4′), 149.22(C(3′)), 143.19(C(2)), 132.94(C(4)), 131.23(C(5)), 129.81(C(1′)), 129.67(C(6)), 120.35(C-3), 120.19 (C-5′), 114.89 (C-1), 110.83 (C-6′), 109.88 (C-2′), 108.49 (C-5′′), 55.97 (C-8′), 55.89 (C-10′), 52.46 (C-9), 26.32 (C-7′′),

25.48 (C-12); HRMS (ESI+) (*m/z*): calcd for $C_{22}H_{22}N_4O_5$ $[M+H]^+$: 422.1585; found: 422.1582.

Methyl 2-acetylamino-5-(2-amino-6-(4-fluorophenyl) pyrimidin-4-yl)benzoate (10)

Colorless solid. Yield 59%; m.p. 240–241 °C; IR (KBr) ν_{max} 3314, 3448, 3501 (H-NH), 3199 (AcN-H), 1690, 1689 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 11.21$ (s, 1H, H-10), 8.84 (d, $J = 8.9$ Hz, 1H, H-3), 8.76 (d, $J = 1.9$ Hz, 1H, H-6), 8.20 (dd, $J_1 = 8.9$ Hz, $J_2 = 1.9$ Hz, 1H, H-4), 8.05 (ddd, $J_1 = 8.7$ Hz, $J_2 = 8.5$ Hz, $J_3 = 2.8$ Hz, 2H, H-2′,6′)), 7.38 (s, 1H, H-5′′)), 7.16 (ddd, $J_1 = 8.7$ Hz, $J_2 = 8.6$ Hz, $J_3 = 2.6$ Hz, 2H, H-3′,5′), 5.18 (s, 2H, H-7′′), 3.98 (s, 3H, H-9)), 2.26 (s, 3H, H-12); ^{13}C NMR (DMSO-d_6, 125 MHz): $\delta_C = 168.77$ (C-11), 167.53 (C-7), 163.95 (C-2′′), 163.90 (C-6′′), 163.70 (d, $J_{C\text{-}F} = 247.7$ Hz, C-4′), 163.33 (C-4′′), 141.39 (C-2), 133.72 (C-1′), 132.36 (C-4), 131.62 (C-5), 129.39 (C-2′,6′), 128.95 (C-6), 120.72 (C-3), 117.56 (C-1), 115.53 C-3′,5′), 101.19 (C-5′′), 52.59 (C-9), 24.76 (C-12); HRMS (ESI+) (*m/z*): calcd for $C_{20}H_{17}N_4FO_3$ $[M+H]^+$: 380.1279; found: 380.1281.

Methyl 2-acetylamino-5-(6-(4-fluorophenyl)-2-methylpyrimidin-4-yl)benzoate (11)

Yield 41% (40% one-pot method *c*); m.p. 178–180 °C; IR (KBr) ν_{max} 3263 (AcN-H), 1701, 1684 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 11.20$ (s, 1H, H-10), 8.85 (d, $J = 8.9$ Hz, 1H, H-3), 8.80 (d, $J = 2.2$ Hz, 1H, H-6), 8.24 (dd, $J_1 = 8.9$ Hz, $J_2 = 2.2$ Hz, 1H, H-4), 8.11 (ddd, $J_1 = 8.8$ Hz, $J_2 = 8.3$ Hz, $J_3 = 2.8$ Hz, 2H, H-2′,6′),7.77 (s, 1H, H-5′′), 7.16 (ddd, $J_1 = 8.8$ Hz, $J_2 = 8.5$ Hz, $J_3 = 2.2$ Hz, 2H, H-3′,5′), 3.98 (s, 3H, H-9), 2.82 (s, 3H, H-7′), 2.25 (s, 3H, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 169.13$(C-11), 168.54 (C-7), 168.38(C-2′′), 164.40 (d, $J_{C\text{-}F} = 251.1$ Hz, C-4′), 163.69 (C-6′′), 163.00 (C-4′′), 143.32 (C-2), 133.34 (C-1′), 132.97 (C-4), 131.05 (C-5), 129.66 (C-6), 129.18 (C-2′,6′), 120.53 (C-3), 115.85 (C-3′,5′), 114.94 (C-1), 108.77 (C-5′′), 52.46 (C-9), 26.30 (C-7′′), 25.46 (C-12); HRMS (ESI+): (*m/z*): calcd for $C_{21}H_{18}N_3FO_3$ $[M+H]^+$: 379.1327; found: 379.1330.

Methyl 2-acetylamino-5-(6-(4-fluorophenyl)-2-phenylpyrimidin-4-yl)benzoate (12)

Colorless needles. Yield 60%; m.p. 207–208 °C; IR (KBr) ν_{max} 3267 (N-H), 1701, 1687 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 11.21$ (s, 1H, H-10), 8.89 (d, $J = 8.8$ Hz, 1H, H-3), 8.85 (d, $J = 2.2$ Hz, 1H, H-6), 8.66 (m, 2H, H-2′′′,6′′′), 8.40 (dd, $J_1 = 8.8$ Hz, $J_2 = 2.2$ Hz, 1H, H-4), 8.26 (ddd, $J_1 = 8.9$ Hz, $J = 8.4$ Гц, $J_3 = 2.8$ Hz, 2H, H-2′,6`), 7.81 (s, 1H, H-5′′), 7.55 (m, 3H, H-3′′′,4′′′,5′′′), 7.25 (m, 2H, H-3′,5′), 4.02 (s, 3H, H-9), 2.29 (s, 3H, H-12); ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta_C = 169.07$ (C-11), 169.30 (C-7), 164.45 (d, $J_{C\text{-}F} = 251.3$ Гц, C-4′), 164.18 (C-2′′), 163.33 (C-6′′), 162.75 (C-4′′), 143.36 (C-2), 137.65 (C-1′′′), 133.23 (C-1′), 132.97 (C-4), 130.88 (C-5), 130.62 (C-4′′′), 129.43 (C-6), 129.12 (C-2′,6′), 128.30 (C-2′′′,6′′′), 128.24 (C-3′′′,5′′′), 120.27 (C-3), 115.73 (C-3′,5′), 114.68 (C-1), 108.78 (C-5′′), 52.48 (C-9), 25.44 (C-12); HRMS (ESI+): (*m/z*): calcd for $C_{26}H_{20}N_3FO_3$ $[M+H]^+$: 441.1483; found: 441.1482.

Methyl 2-acetylamino-5-(6-(4-methoxyphenyl)-2-(pyridin-3-yl)pyrimidin-4-yl)benzoate (13)

Colorless needles. Yield 30%; m.p. 213–215 °C; IR (KBr) ν_{max} 3273 (N-H), 1701, 1687 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 11.24$ (s, 1H, H-10), 9.86 (d, $J = 1.2$ Hz, 1H, H-2′′′), 8.93 (d, $J = 2.2$ Hz, 1H, H-6), 8.90 (d, $J = 8.9$ Hz, 1H, H-3), 8.89 (dt, $J_1 = 8.0$ Hz, $J_2 = 1.2$ Hz, 1H, H-4′′′), 8.73 (dd, $J_1 = 5.8$ Hz, $J_2 = 1.2$ Hz, 1H, H-6′′′), 8.38 (dd, $J_1 = 8.9$ Hz, $J_2 = 2.2$ Hz, 1H, H-4), 8.24 (d, $J = 8.8$ Hz, 2H, H-2′,6′), 7.91 (s, 1H, H-5′′), 7.45 (ddd, $J_1 = 8.0$ Hz, $J_2 = 5.8$ Hz, $J_3 = 1.2$ Hz, 1H, H-5′′′), 7.06 (d, $J = 8.0$ Hz, 2H, H-3′,5′), 4.02 (s, 3H, H-9), 3.90 (s, 3H, H-8′), 2.28 (s, 3H, H-12); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ_C: 169.14 (C-11), 168.43 (C-7), 164.38 (C-6′′), 162.84 (C-4′′), 162.56 (C-2′′), 162.10 (C-4′), 151.13 (C-6′′′), 150.09 (C-2′′′), 143.50 (C-2), 135.56 (C-4′′′), 133.48 (C-3′′′), 132.96 (C-4), 130.96 (C-5), 129.68 (C-6), 129.92 (C-1′), 128.73 (C-2′,6′), 123.19 (C-5′′′), 120.45 (C-3), 114.96 (C-1), 114.24 (C-3′,5′), 109.07 (C-5′′), 55.36 (C-8′), 52.59 (C-9), 25.50 (C-12); HRMS (ESI+) (*m/z*): calcd for $C_{26}H_{22}N_4O_4$ $[M+H]^+$: 454.1636; found: 454.1641.

Methyl 2-acetylamino-5-(6-(4-fluorophenyl)-2-(pyridin-3-yl) pyrimidin-4-yl)benzoate (14)

Colorless needles. Yield 50%; m.p. 227–229 °C. IR (KBr) ν_{max} 3259 (N-H), 1697, 1685 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ_H: 11.18 (s, 1H, H-10), 9.77 (s, 1H, H-2′′′), 8.83 (d, $J = 8.9$ Hz, 1H, H-3), 8.81 (d, $J = 1.9$ Hz, 1H, H-6), 8.78 (d, $J = 7.9$ Hz, 1H, H-4′′′), 8.70 (d, $J = 5.5$ Hz, 1H, H-6′′′), 8.28 (dd, $J_1 = 8.9$ Hz, $J_2 = 1.9$ Hz, 1H, H-4), 8.18 (ddd, $J_1 = 8.6$ Hz, $J_2 = 8.5$ Гц, $J_3 = 2.9$ Hz, 2H, H-2′,6′), 7.79 (s, 1H, H-5′′)), 7.39 (dd, $J_1 = 7.9$ Hz, $J_2 = 5.5$ Hz, $J_3 = 1.2$ Hz, 1H, H-5′′′)), 7.18 (ddd, $J_1 = 8.6$ Hz, $J_2 = 8.3$ Hz, $J_3 = 2.5$ Hz, 2H, H-3′,5′), 3.98 (s, 3H, H-9), 2.24 (s, 3H, H-12); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: 169.14 (C-11), 168.25 (C-7), 164.62(d, $J_{C\text{-}F} = 252.0$ Hz, C-4′), 163.55 (C-6′′), 162.92 (C-4′′), 162.51(C-2′′), 151.21 (C-6′′′), 149.97 (C-2′′′), 143.61 (C-2), 135.46 (C-4′′′), 133.15 (C-3′′′), 132.81 (C-4), 132.77 (C-1′), 130.38 (C-5), 129.54 (C-6), 129.15 (C-2′,6′), 123.18 (C-5′′′), 120.39 (C-3), 115.88

(C-3′,5′), 114.83 (C-1), 109.26 (C-5′′), 52.59 (C-9), 25.46 (C-12); HRMS (ESI+) (*m/z*): calcd for $C_{25}H_{19}N_4FO_3$ $[M+H]^+$: 442.1436; found: 442.1434.

Methyl 2-acetylamino-5-(6-(4-fluorophenyl)-[2,2'-bipyrimidin]-4-yl)benzoate (15)

Colorless needles. Yield 62%; m.p. 119–121 °C; IR (KBr) ν_{max} 3309 (N-H), 1698, 1689 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): $\delta = 11.24$ (s, 1H, H-10), 9.05 (d, $J =$ 4.8 Hz, 2H, H-4′′′,6′′′), 8.92 (d, $J = 1.9$ Hz, 1H, H-6), 8.90 (d, $J = 8.9$ Hz, 1H, H-3), 8.41 (dd, $J_1 = 8.9$ Hz, $J_2 = 1.9$ Hz, 1H, H-4), 8.27 (ddd, $J_1 = 8.6$ Hz, $J_2 = 8.2$ Hz, $J_3 = 2.6$ Hz, 2H, H-2′,6′)), 8.10 (s, 1H, H-5′′), 7.45 (t, $J = 4.8$ Hz, 1H, H-5′′′), 7.22 (ddd, $J_1 = 8.6$ Hz, $J_2 = 8.5$ Hz, $J_3 = 2.5$ Hz, 2H, H-3′,5′), 3.98 (s, 3H, H-9), 2.27 (s, 3H, H-12); ^{13}C NMR ($CDCl_3$,125 MHz): $\delta = 169.18$ (C-11), 168.37 (C-7), 164.91 (C-2′′), 164.69 (d, $J_{C\text{-}F} = 251.4$ Hz, C-4′), 164.37 (C-2′′′), 163.28 (C-6′′), 163.25 (C-4′′), 157.85 (C-4′′′,6′′′), 143.68 (C-2), 133.46 (C-4), 132.89 (C-1′), 130.63 (C-5), 130.13 (C-6), 129.68 (C-2′,6′), 121.16 (C-5′′′), 120.53 (C-3), 115.98 (C-3′,5′), 115.00 (C-1), 111.91 (C-5′′), 52.48 (C-9), 25.51 (C-12); HRMS (ESI+) (*m/z*): calcd for $C_{24}H_{18}N_5FO_3$ $[M+H]^+$: 443.1388; found: 443.1394.

Methyl 2-acetylamino-5-(6-(4-methoxyphenyl)-[2,2'-bipyrimidin]-4-yl)benzoate (16)

Yellow solid. Yield 32%; m.p. 240–241 °C; IR (KBr) ν_{max}: 3311 (N-H), 1698, 1689 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 11.23$ (s, 1H, H-10), 9.04 (d, $J = 4.8$ Hz, 2H, H-4′′′, 6′′′), 8.92 (d, $J = 2.3$ Hz, 1H, H-6), 8.89 (d, $J =$ 8.9 Hz, 1H, H-3), 8.39 (dd, $J_1 = 8.9$ Hz, $J_2 = 2.3$ Hz, 1H, H-4), 8.25 (d, $J = 8.8$ Hz, 2H, H-2′,6′), 8.08 (s, 1H, H-5′′), 7.44 (t, $J = 4.8$ Hz, 1H, H-5′′′), 7.03 (d, $J = 8.8$ Hz, 2H, H-3′,5′), 3.97 (s, 3H, H-9), 3.88 (s, 3H, H-8′), 2.26 (s, 3H, H-12); ^{13}C-NMR ($CDCl_3$, 125 MHz): $\delta = 169.17$ (C-11), 168.44 (C-7), 165.42 (C-2′′), 163.97 (C-2′′′), 163.48 (C-6′′), 163.17 (C-4′′), 162.12 (C-4′), 157.81 (C-4′′′,6′′′), 143.38 (C-2), 133.44 (C-4), 130.97 (C-5), 130.09 (C-6), 129.14 (C-2′,6′), 129.05 (C-1′), 121.06 (C-5′′′), 120.47 (C-3), 114.97 (C-1), 114.22 (C-3′,5′), 111.34 (C-5′′), 55.35(C-8′), 52.46 (C-9), 25.51(C-12); HRMS (ESI+) (*m/z*): calcd for $C_{25}H_{21}N_5O_4$ $[M+H]^+$: 455.1588; found: 455.1581.

Methyl 2-acetylamino-5-(6-phenyl-[2,2'-bipyrimidin]-4-yl) benzoate (17)

Yellow solid. Yield 35%; m.p. 214–216 °C; IR (KBr) ν_{max} 3259 (N-H), 1701, 1682 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 11.25$ (s, 1H, H-10), 9.05 (d, $J = 4.8$ Hz, 2H, H-4′′′,6′′′), 8.94 (d, $J = 2.2$ Hz, 1H, H-6), 8.91 (d, $J =$ 8.8 Hz, 1H, H-3), 8.42 (dd, $J_1 = 8.8$ Hz, $J_2 = 2.2$ Hz, 1H, H-4), 8.25 (m, 2H, H-2′,6′)), 8.15 (s, 1H, H-5′′), 7.54 (m, 3H, H-3′,4′,5′), 7.45 (t, $J = 4.8$ Hz, 1H, H-5′′′),3.98 (s, 3H, H-9)), 2.27 (s, 3H, H-12); ^{13}C-NMR ($CDCl_3$, 125 MHz): $\delta =$ 169.09 (C-11), 168.33 (C-7), 166.03 (C-2′′), 164.20 (C-2′′′), 163.31 (C-6′′), 163.19 (C-4′′), 157.77 (C-4′′′,6′′′), 143.54 (C-2), 136.68 (C-1′), 133.42 (C-4), 130.94 (C-4′), 130.69 (C-5), 130.06 (C-6), 128.83 (C-2′,6′), 127.49 (C-3′,5′), 121.03 (C-5′′′), 120.45 (C-3), 114.92 (C-1), 112.27 (C-5′′), 52.40 (C-9), 25.45 (C-12); HRMS (ESI+) (*m/z*): calcd for $C_{24}H_{19}N_5O_3$ $[M+H]^+$: 425.1482; found: 425.1481.

(E)-Methyl 2-acetylamino-5-(6-(2,3-dimethoxystyryl)-2-methylpyrimidin-4-yl)benzoate (18)

Yellow needles. Yield 64%; m.p. 216–217 °C; IR (KBr) ν_{max}: 3259 (N-H), 1701, 1687 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$,400 MHz): $\delta = 11.20$ (s, 1H, H-10), 8.86 (d, $J =$ 8.8 Hz, 1H, H-3), 8.80 (d, $J = 2.2$ Hz, 1H, H-6), 8.25 (dd, $J_1 = 8.8$ Hz, $J_2 = 2.2$ Hz, 1H, H-4), 8.14 (d, $J = 16.2$ Гц, 1H, H-9′′)), 7.54 (s, 1H, H-5′′), 7.27 (dd, $J_1 = 7.8$ Hz, $J_2 =$ 1.3 Hz, 1H, H-6′), 7.17 (d, $J = 16.2$ Hz, 1H, H-8′′), 7.07 (dd, $J_1 = 8.0$ Hz, $J_2 = 7.8$ Hz, 1H, H-5′), 6.91 (dd, $J_1 = 8.0$ Hz, $J_2 = 1.3$ Hz, 1H, H-4′), 3.99 (s, 3H, H-9), 3.90 (s, 3H, H-8′), 3.88 (s, 3H, H-10′), 2.80 (s, 3H, H-7′′), 2.26 (s, 3H, H-12); ^{13}C-NMR ($CDCl_3$, 125 MHz): $\delta = 168.76$ (C-11), 168.13 (C-7), 167.94 (C-2′′), 163.08 (C-6′′), 162.41 (C-4′′), 152.70 (C-2′), 147.57 (C-3′), 142.90 (C-2), 132.67 (C-4), 131.08 (C-9′′), 130.88 (C-5), 129.58 (C-1′), 129.35 (C-6), 127.58 (C-8′′), 123.75 (C-5′), 120.11 (C-3), 118.56 (C-6′), 114.64 (C-1), 112.60 (C-4′), 110.09 (C-5′′), 60.87 (C-8′), 55.44 (C-10′), 52.09 (C-9), 25.92 (C-7′′), 25.13 (C-12); HRMS (ESI+) (*m/z*): calcd for $C_{25}H_{25}N_3O_5$ $[M+H]^+$: 447.1789; found: 447.1743.

(E)-Methyl 2-acetylamino-5-(6-(2,3-dimethoxystyryl)-2-phenylpyrimidin-4-yl)benzoate (19)

Yellow needles. Yield 56%; m.p. 204–205 °C; IR (KBr) ν_{max} 3279 (N-H), 1701, 1687 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): $\delta = 11.23$ (s, 1H, H-10), 8.91 (d, $J =$ 2.2 Hz, 1H, H-6), 8.89 (d, $J = 8.8$ Hz, 1H, H-3), 8.65 (m, 2H, H-2′′′,6′′′), 8.43 (dd, $J_1 = 8.8$ Hz, $J_2 = 2.2$ Hz, 1H, H-4), 8.32 (d, $J = 16.1$ Hz, 1H, H-9′′), 7.60 (s, 1H, H-5′′), 7.48–7.56 (m, 3H, H-3′′′,4′′′,5′′′), 7.32 (dd, $J_1 = 8.0$ Hz, J_2 $= 1.0$ Hz, 1H, H-6′), 7.27 (d, $J = 16.1$ Hz, 1H, H-8′′), 7.10 (dd, $J_1 = 8.0$ Hz, $J_2 = 7.6$ Hz, 1H, H-5′), 6.93 (dd, $J_1 = 7.6$ Hz, $J_2 = 1.0$ Hz, 1H, H-4′), 4.01 (s, 3H, H-9), 3.93 (s, 3H, H-8′), 3.89 (s, 3H, H-10′), 2.27(s, 3H, H-12); ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta = 169.12$ (C-11), 168.50 (C-7), 164.26 (C-2′′), 163.51 (C-6′′), 162.77 (C-4′′), 153.07 (C-2′), 147.95 (C-3′), 143.34 (C-2), 137.95 (C-1′′′), 133.16 (C-4), 131.45 (C-9′′), 131.27 (C-5), 130.51 (C-4′′′), 130.03 (C-

1′), 129.64 (C-6), 128.35 (C-3‴,5‴), 128.33 (C-(2‴,6‴), 128.01 (C-8′), 124.12 (C-5′), 120.43 (C-3), 119.00 (C-6′), 114.86 (C-1), 112.90 (C-4′), 111.38 (C-5″), 61.24 (C-8′), 55.79 (C-10′), 52.53 (C-9), 25.51 (C-12); HRMS (ESI+) (*m/z*): calcd for $C_{30}H_{27}N_3O_5$ $[M+H]^+$: 509.1945; found: 509.1946.

(E)-Methyl 2-acetylamino-5-(6-(2,5-dimethoxystyryl)-2-methylpyrimidin-4-yl)benzoate (20)

Yellow needles. Yield 42%; m.p. 197–199 °C; IR (KBr) ν_{max} 3271 (N-H), 1703, 1686 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 11.20$ (s, 1H, H-10), 8.85 (d, $J =$ 8.8 Hz, 1H, H-3), 8.81 (d, $J = 2.3$ Hz, 1H, H-6), 8.24 (dd, $J_1 = 8.8$ Hz, $J_2 = 2.3$ Hz, 1H, H-4), 8.14 (d, $J = 16.2$ Hz, 1H, H-9″), 7.55 (s, 1H, H-5″), 7.18 (d, $J = 2.7$ Hz, 1H, H-6′), 7.17 (d, $J = 16.2$ Hz, 1H, H-8″), 6.88 (dd, $J_1 = 8.6$ Hz, $J_2 = 2.7$ Hz, 1H, H-4′), 6.87 (d, $J = 8.6$ Hz, 1H, H-3′), 3.99 (s, 3H, H-9), 3.88 (s, 3H, H-8′), 3.80 (s, 3H, H-10′), 2.80 (s, 3H, H-7″), 2.26 (s, 3H, H-12); ^{13}C NMR ($CDCl_3$,125 MHz): $\delta = 169.10$ (C-11), 168.48 (C-7), 168.22 (C-2″), 163.57 (C-6″), 162.65 (C-4″), 153.53 (C-5′), 152.26 (C-2′), 143.13 (C-2), 132.99 (C-4), 131.70 (C-9″), 131.27 (C-5), 129.68 (C-6), 127.15 (C-8″), 125.24 (C-1′), 120.40 (C-3), 116.08 (C-4′), 114.95 (C-1), 112.31 (C-6′)), 112.27 (C-3′), 110.30 (C-5″), 56.02 (C-8′), 55.68 (C-10′), 52.42 (C-9)), 26.26 (C-7″), 25.47 (C-12); HRMS (ESI+): (*m/z*): calcd for $C_{25}H_{25}N_3O_5$ $[M+H]^+$: 447.1789; found: 447.1783.

(E)-Methyl 2-acetamido-5-(6-(3,4-dimethoxystyryl)-2-methylpyrimidin-4-yl)benzoate (21)

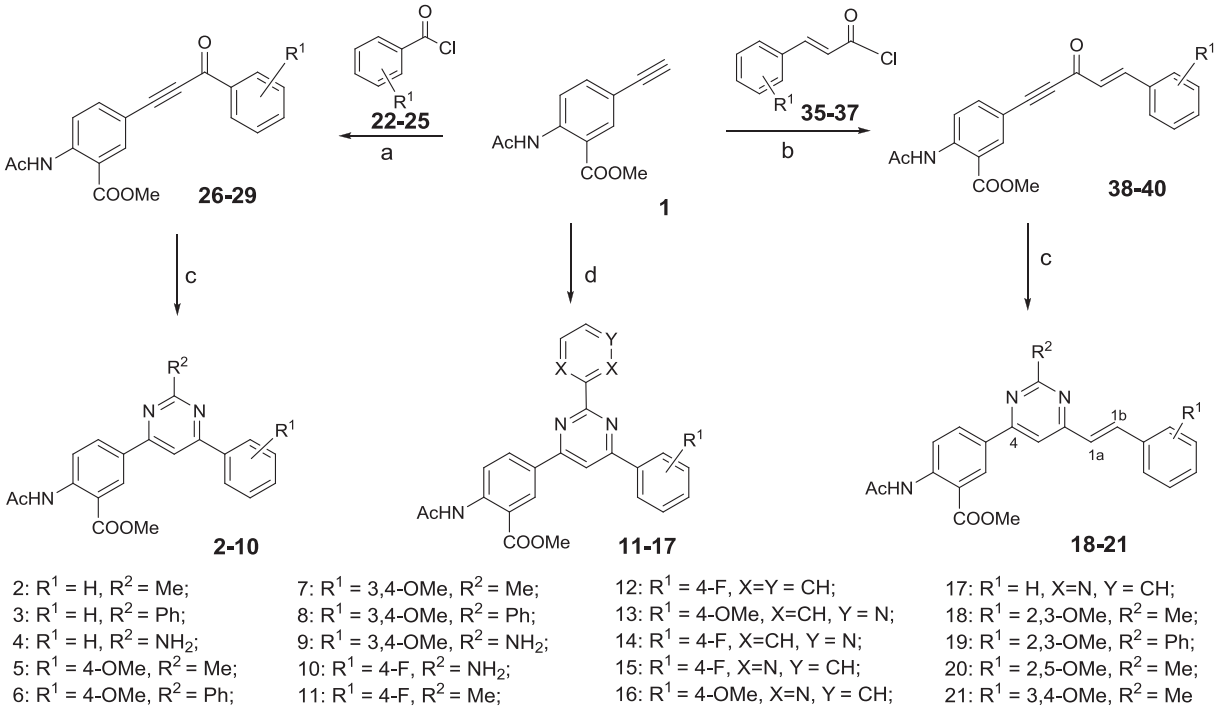
Yellow needles. Yield 65%; m.p. 217–219 °C; IR (KBr) ν_{max} 3265 (N-H), 1703, 1687 (C=O) cm^{-1}. ^1H-NMR ($CDCl_3$, 400 MHz): $\delta = 11.20$ (s, 1H, H(10)), 8.85 (d, $J =$ 8.7 Hz, 1H, H(3)), 8.80 (d, $J = 2.1$ Hz, 1H, H(6)), 8.22 (dd, $J_1 = 8.7$ Hz, $J_2 = 2.1$ Hz, 1H, H(4)), 7.82 (d, $J = 16.0$ Hz, 1H, H(9″)), 7.48 (s, 1H, H(5″)), 7.21–7.15 (m, 2H, H (2′,6′)), 6.99 (d, $J = 16.0$ Hz, 1H, H (8″)), 6.88 (d, $J = 8.8$ Hz, 1H, H(5′)), 3.98 (s, 3H, H(9)), 3.93 (s, 3H, H(10′)), 3.91 (s, 3H, H(8′)), 2.79 (s, 3H, H(7″)), 2.26 (s, 3H, H(12)); ^{13}C NMR ($CDCl_3$,125 MHz): $\delta = 169.05$ (C-11), 168.37 (C-7), 168.10 (C-2″), 163.14 (C-6″), 162.57 (C-4″), 150.17 (C-3′), 149.02 (C-4′), 143.13 (C-2), 136.47 (C-6′), 132.85 (C-4), 131.10 (C-5), 129.56 (C-6), 128.61 (C-1′), 124.08 (C-9″), 121.62 (C-8″), 120.32 (C-3), 114.87 (C-1), 110.92 (C-2′), 110.17 (C-5′), 109.11 (C-5″), 55.77 (C-10′), 55.69 (C-8′), 52.37 (C-9), 26.21 (C-7″), 25.41 (C-12); HRMS (ESI+) (*m/z*): calcd for $C_{25}H_{25}N_3O_5$ $[M+H]^+$: 447.1789; found: 447.1742.

Cell culture and cytotoxicity assay

The human cancer cells of the CEM-13 (the cells of T-cellular human leucosis), U-937 (human monocytes), MDA-MB-231 and BT-474 (breast cancer cell lines) and DU-145 (prostate cancer) were used in this study. The cells were cultured in the RPMI-1640 medium that contained 10% embryonic calf serum, L-glutamine (2 mmol/L), gentamicin (80 lg/ml), and lincomycin (30 mg/ml) in a CO_2 incubator at 37 °C. The tested compounds were dissolved in DMSO and added to the cellular culture at the required concentrations. Three wells were used for each concentration. The cells which were incubated without the compounds were used as a control. Cells were placed on 96-well microliter plates and cultivated at 37 °C C in 5% CO_2/95% air for 72 h. The cell viability was assessed through an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-phenyl-2H-tetrazolium bromide] conversion assay. 1% MTT was added to each well. Four hours later DMSO was added and mixed for 15 min. Optical density (*D*) of the samples was measured on a BioRad 680 multi-well spectrophotometer (USA) at the wavelength of 570 nm. The 50% cytotoxic dose (CTD_{50}) of each compound (i.e., the compound concentration that causes the death of 50% of cells in a culture, or decreases the optical density twice as compared to the control wells) was calculated from the data obtained. Statistical processing of the results was performed using the Microsoft Excel-2007, STATISTICA 6.0, and GraphPad Prism 5.0 programs. The results are given as an average value ± a deviation from the average. Reliability of differences (p) was estimated using the Student t test. The differences with $p < 0.05$ were considered as reliable. The experimental results are given as the data average values obtained from three independently conducted experiments.

Molecular modeling study

Molecular modeling was carried out in the Schrodinger Maestro visualization environment using applications from the Schrödinger Small Molecule Drug Discovery Suite 2017-1 package (Canvas 2015). Three-dimensional structures of the derivatives were obtained empirically in the LigPrep application using the OPLS3 force field (Harder et al. 2016). All possible tautomeric forms of compounds, as well as various states of polar protons of molecules in the pH range of 7.0 ± 2.0 were taken into account. A search was made for X-ray crystallographic models of CDK6 and CDK9 in the PDB database. A set of models of the team of authors who studied the binding of known inhibitors abemaciclib, palbociclib and ribociclib to CDK6 was selected (Chen et al. 2016). Model with PDB ID 5L2T (resolution of 2.37 Å) was used for the



Scheme 1 Synthesis of 2,4,6-trisubsuituted pyrimidines

calculations. To simulate the possible mechanism of CDK6 and (or) CDK9 inhibition, molecular docking of new compounds to the binding site of known inhibitors has been performed in the Glide application. The search area for docking was selected automatically, based on the size and physicochemical properties of ribociclib (for CDK6). The extra precision (XP) algorithm of docking was applied. Docking was performed in comparison with the known inhibitor Ribociclib. The three-dimensional structures of inhibitors were obtained in the PubChem database and prepared in the LigPrep application. Non-covalent interactions of compounds in the binding site were visualized using Biovia Discovery Studio Client (DS Client 2015).

Results and discussion

Chemistry

The 2,4,6-trisubstituted pyrimidines **2**–**21**, which are exemplified in the present article, were prepared starting from methyl 2-(*N*-acetylamino)-5-ethynylbenzoate **1** (Osadchii et al. 2007), as outlined in Scheme 1. The cross-coupling under Sonogashira reaction conditions of the alkyne **1** with benzoic acid chlorides **22**–**25** gave access to key intermediates **26**–**29** isolated after column chromatography and crystallization as solids in the yield 51–82%. Subsequently, compounds **22**–**25** were condensed with amidine hydrochlorides **30**–**31** or guanidine carbonate **32** in the presence of Na_2CO_3 in MeCN to afford the target compounds **2**–**11** (yield 41–82%). Electron donating groups in the aroyl chloride reduced the yield of the target pyrimidines, and withdrawing groups—increased it. For providing a most powerful approach to pyrimidines **2**–**21** by limited number of reaction steps, we assumed that two-step Pd-catalyzed Sonogashira coupling and subsequent condensation could be carried out in a one-pot manner (in multicomponent reaction conditions) (Willy and Müller 2008, 2009]. The use of a bimetallic catalyst system (the presence of CuI) and the necessity of employing an excess of triethylamine (2 equiv) create the conditions for the formation of a dimer product from the terminal alkyne **1**. In this regard, it was necessary to change the reaction conditions in the multicomponent sequence. An effective route to alkynyl ketones based only on a palladium catalytic system, which give simultaneously the possibility to change the

Table 1 Cytotoxicity of 2,4,6-trisubstituted pyrimidines **2–4**, **6–21**

Compound	Cytotoxicity (GI_{50}, μM) against cell lines[a]				
	CEM-13	U-937	MDA-MB-231	BT-474	DU-145
2	>200	>200	>200	>200	>200
3	65.32 ± 10.81	112.62 ± 5.48	42.14 ± 3.54	39.26 ± 2.12	51.12 ± 6.18
4	39.16 ± 2.18	38.12 ± 3.26	33.42 ± 1.96	35.68 ± 2.38	32.36 ± 2.06
6	>200	186,37 ± 17,55	35,06 ± 4,22	28,26 ± 4,51	176,6 ± 21,83
7	>200	>200	>200	>200	>200
8	104,20 ± 13,13	>200	>200	>200	>200
9	36.33 ± 6,76	10.15 ± 1.49	>200	>200	>200
10	43.22 ± 5.71	21.18 ± 5.11	23.11 ± 3.58	20.26 ± 3.63	47.21 ± 3.82
11	66.32 ± 2.34	48.64 ± 2.18	28.16 ± 1.36	42.45 ± 2.62	36.28 ± 2.44
12	59.18 ± 3.14	34.26 ± 1.56	36.18 ± 3.08	29.21 ± 1.56	31.76 ± 1.78
13	36.28 ± 1.68	12.12 ± 1.46	26.18 ± 2.13	23.55 ± 1.82	108.19 ± 16.32
14	28.54 ± 1.56	15.18 ± 2.32	19.11 ± 1.72	14.47 ± 1.63	85.48 ± 12,08
15	13.12 ± 0.38	31.22 ± 2.62	25.32 ± 1.61	12.18 ± 0.88	17.36 ± 0.96
16	18.26 ± 0.88	23.38 ± 2.18	15.48 ± 1.12	12.18 ± 1.28	32.18 ± 16.66
17	21.42 ± 1.26	23.46 ± 1.42	12.66 ± 0.92	10.48 ± 0.64	29.48 ± 2.76
18	30.26 ± 2.52	31.11 ± 2.52	10.44 ± 0.66	19.48 ± 1.54	11.25 ± 1.08
19	31.18 ± 1.62	38.58 ± 3.12	11.28 ± 1.16	14.56 ± 1.72	10.18 ± 1.44
20	38.21 ± 2.34	33.12 ± 4.64	10.08 ± 1.12	20.16 ± 1.62	12.14 ± 1.36
21	48.16 ± 5.32	35.42 ± 2.78	16.36 ± 1.28	12.48 ± 0.92	11.82 ± 0.66
Doxorubicin	3.4 ± 1.08	0.2 ± 0.06	7.91 ± 0.51	2.63 ± 0.78	6.61 ± 0.34

[a]GI_{50}: concentration at which 50% growth inhibition of tumor cells is observed after 72 h incubation

solvents from esters to the less Lewis-basic solvents, consist in the employing of the most practical Beller's ligand [di(1-adamantyl)benzylphosphonium bromide] and $PdCl_2$ as the source of palladium. This copper-free system was previously employed in Sonogashira coupling (Köllhofer et al. 2003, Nordmann et al. 2013). Using catalytic system $PdCl_2$ – $(1\text{-Ad})_2PBn{\cdot}HBr$ and carrying out the reaction of alkyne **1** with 4-fluorobenzoyl chloride **25** in dichloromethane for 2 h (TLC-control), evaporation the solvent and subsequent condensation of the crude reaction mixture with amidines **30** or **31** afforded pyrimidines **11**, **12** (40–60% isolated yield). The one-put copper-free procedure was also used for the synthesis of substituted bis-heterocyclic systems (2-(pyridine-3-yl)-pyrimidines and 2,2'-bipyrimidines) **13–17** from compound **1**, aroyl chlorides **22**, **23**, **25** and amidine hydrochlorides **33, 34**. We found that addition of amidine hydrochloride in methanol solution and using of NEt_3 as a base was more efficient (30–62% isolated yield by two step) than using of Na_2CO_3 as the base and carry out the reaction in MeCN (7–19% isolated yield by two step). Synthesis of substituted (2-(pyridine-3-yl)-pyrimidines and 2,2'-bipyrimidines) was of interest in the aim of construction of new compounds for control of cell proliferation (Hu et al. 2006) or analogs of Bosentan, the first-in-class drug used in treatment of pulmonary arterial hypertension (Lepri et al. 2016).

We also prepared a series of styryl substituted pyrimidines **18–21** by the reaction of alkyne **1** with *trans*-cinnamoyl chlorides **35–37**, and subsequent condensation of the obtained en-in-unsaturated ketones **38–40** with amidine hydrochlorides **30**, **31**. We found, that the yield of compounds **38–40** depends on the reaction conditions: the coupling of **1** with cinnamoyl chloride **35** under Sonogashira reaction conditions gave compound **38** in the isolated yield 27% (in additional dimer of compound 1 was obtained). The yield of **38** was increased up to 55% by using of $PdCl_2$ (1.5 mol %),-$(1\text{-Ad})_2PBn{\bullet}HBr$ (3 mol %) as a catalyst, NEt_3 as the base (1.2 eq) and CH_2Cl_2 as the solvent. The subsequent pyrimidines **18–21** were obtained in the yield 42–65% by boiling in MeCN in the presence of Na_2CO_3. The so introduction of a styryl moiety in the pyrimidine core is of interest because of the potency of obtained compounds for search of novel anti-cancer agents (Tong et al. 2016).

The chemical structures of the target compounds were confirmed by IR, NMR, and mass spectrometry (MS spectra). The IR spectra of compounds 26–29 and 38–40 are featured by the presence of the absorption band of the alkyne linker group at 2191–2197 cm^{-1}. The ^1H and ^{13}C NMR spectra of all synthesized pyrimidines agree well with their structure and contain the characteristic signals of pyrimidine ring and the corresponding substituents. The

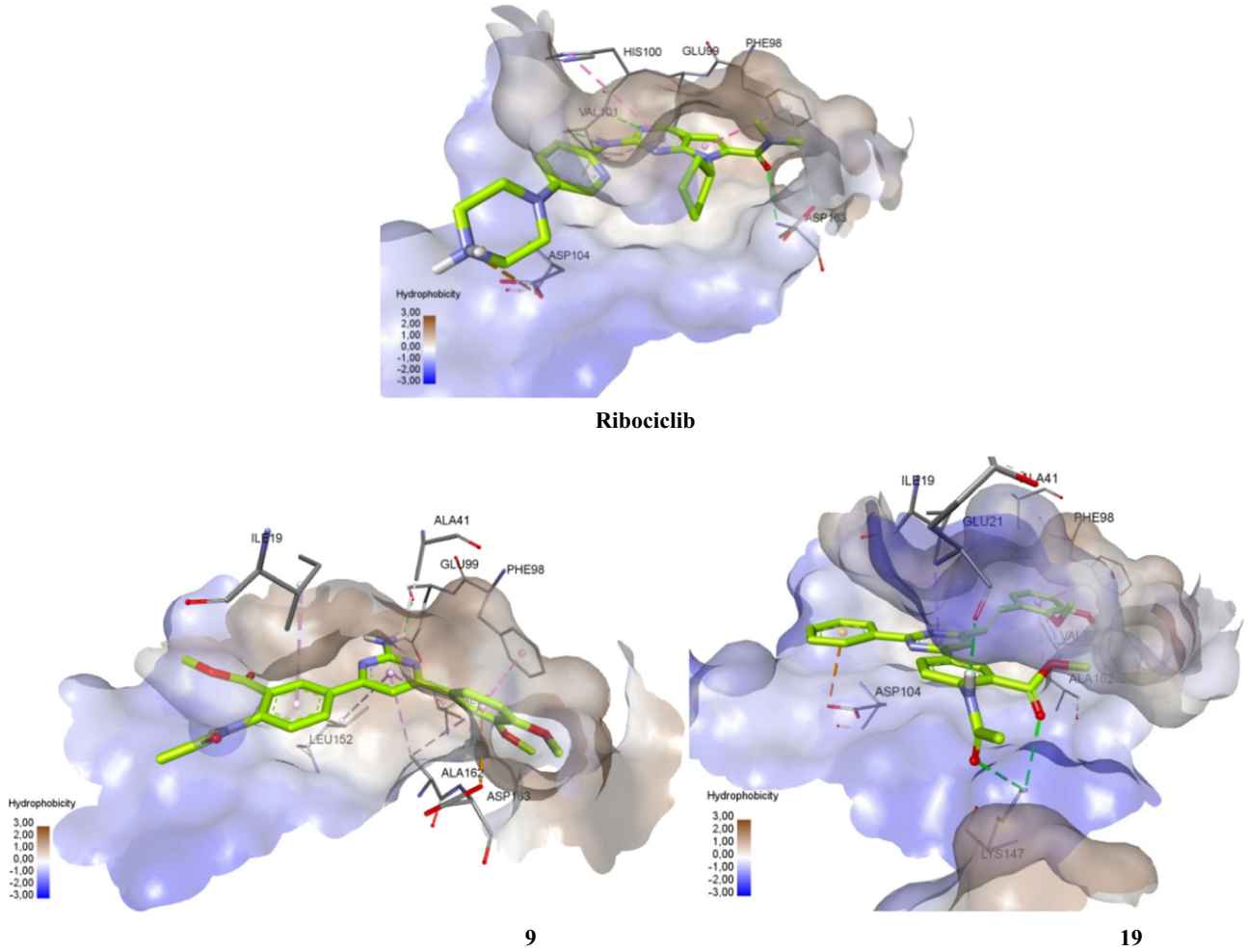


Fig. 2 Non-covalent interaction of novel pyrimidine derivatives **9** and **19** in the binding site CDK6 compared with Ribociclib (the hydrophobic interactions not shown). The interactions are shown by dashed lines: green-hydrogen bonds, orange – electrostatic interactions, purple – hydrophobic interactions and interactions of π-systems

spin-spin coupling constants between alkenyl proton signals H-1a and H-1b (*J* 16.0–16.2 Hz) indicate the formation of compounds 18–21 with the (*E*)-configuration of the double bond. The position of the double bond at the newly introduced aryl substituent was determined using the two-dimensional ^{1}H–^{13}C correlation technique (HMBC). The *C*2 carbon atom of the 3,4-dimethoxyphenyl substituent in the HNBC spectra of compound 20 (δC 152.26 ppm) was found to interact with 1b-H (δ 8.14 ppm), the *C*5 carbon atom of the anthranyl substituent (δC 131.27 ppm) was found to interact with H-3 proton of the indicated substituent (δ 8.85 ppm), and also with H-5 proton of the pyrimidine ring (δ 7.55 ppm). The proton H-5 was also coupled with 1a-*C*H = (δ 127.15). These data indicated that the anthranyl and (2,5-dimethoxy)styryl substituents are attached to C4 and C6, respectively (See also Suppl. Part).

So, we prepared original 2,4,6-substituted pyrimidines corresponding to the amino, pyridine or pyrimidine substitution in position 2, aryl or styryl substitution in position 4, and anthranylic acid substitution in position 6 (Scheme 1). All of these compounds were tested for their cytotoxic activity against five cancer cell lines and were studied as cyclin-dependent kinase CDK6 and CDK9 inhibitors using docking experiments.

Biological evaluation

The cytotoxic activity of the synthesized pyrimidines with methylanthranilate substituent in the 6 position **2–4, 6–21** was determined by measuring the concentration inhibiting human tumor cell viability by 50% (GI_{50}). Doxorubicin, which is one of the most effective anticancer agents, was used as the reference drug in this study. The GI_{50} was determined using the conventional MTT assay, which allows to estimate the number of survived cells spectrophotometrically (Wilson et al. 1990). The results are presented in Table 1. From the results (Table 1) it can be seen, that despite to the variation in biological activity between

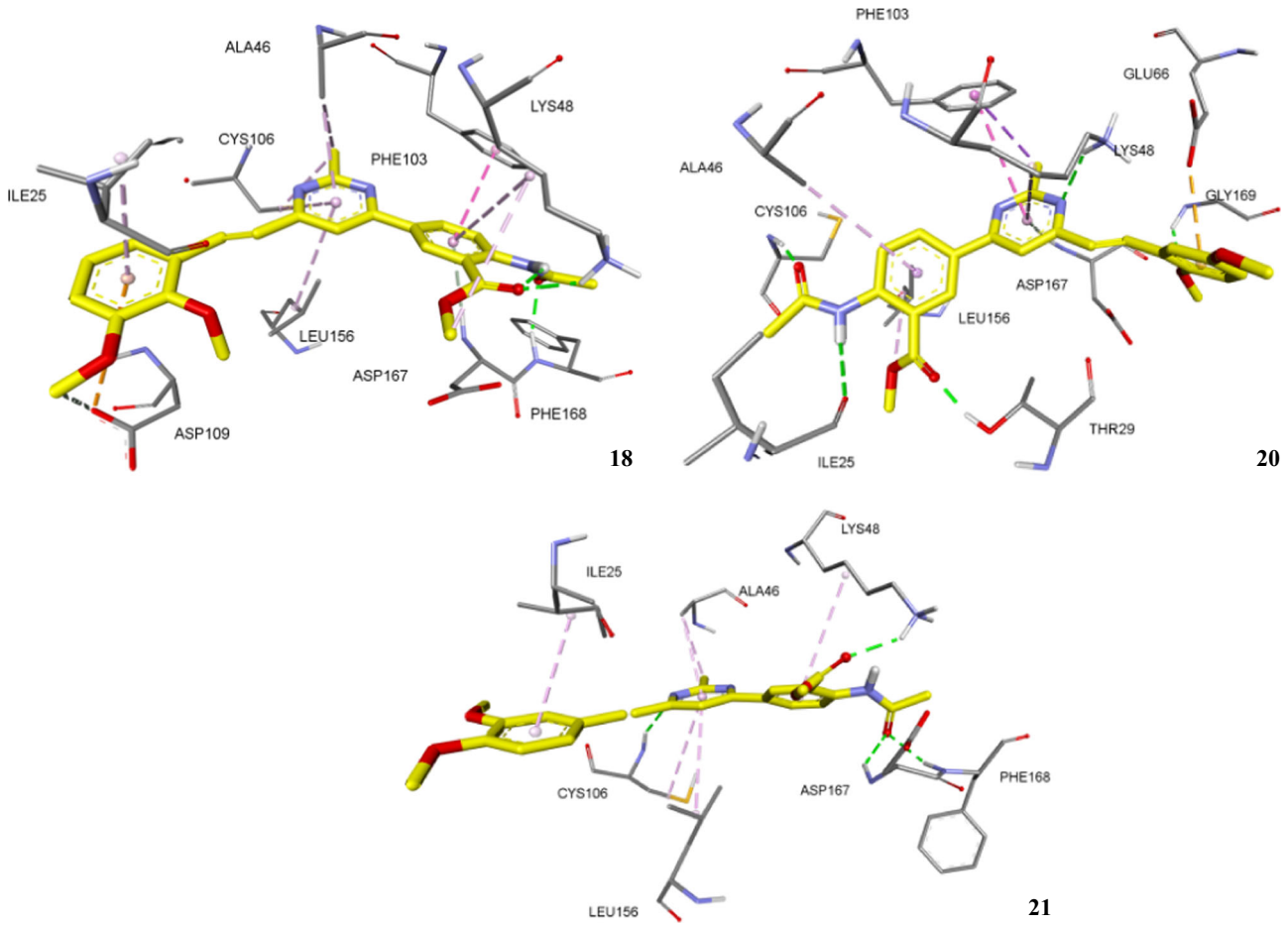


Fig. 3 Non-covalent interaction of novel pyrimidine derivatives **18**, **20** and **21** in the CDK9 binding site. The interactions are shown by dashed lines: green-hydrogen bonds, orange-electrostatic interactions, purple-hydrophobic interactions, and π-system interactions

the compounds was not very high-yet, the following points can still be concluded. At first glance, it is evident that the substituent in the 2- and 6- positions in the pyrimidine ring are essential for the cytotoxicity of the 4-antranylate substituted pyrimidines. Studying the comparable effect of the compounds on viability of human cancer lines revealed that compounds **10**, **14**–**21** demonstrated the increase of potency against breast cancer cell lines MDA-MB-231 and BT-474. 2-Aminopyrimidine **10**, with a 6-fluorophenyl substituent demonstrated an increasing of potency compared with 6-phenyl- or 6-(3,4-dimethoxyphenyl)-substituted compounds **4** and **9** on the breast cancer cell lines. Characteristically, 2-aminopyrimidine **9** shown selectivity on the human monocyte-like cells U-937. The heterocyclic (pyridine or pyrimidine ring) substituent in the 2 position seems to have an important role in the cytotoxic activity and potency (compounds **13**–**17**). A remarkable increase in activity and selectivity towards lymphoid cancer cell line U-937 was observed for compounds **13** and **14** containing the pyridine substituent in the 2 position of the pyrimidine ring. Tri-substituted pyrimidines **18**–**21**, containing a (*E*)-styryl substituent in the 6 position of the pyrimidine core, demonstrated selectivity towards breast cancer cel lines and prostate cancer cell lines DU-145. Compounds **18** and **20** shown the best potency towards the breast cancer cell lines MDA-MB-231; theirs activity was comparable with the activity of Doxorubicin on this cell lines.

Molecular modeling

Cyclin-dependent kinases (CDKs), a family of serine/threonine protein kinases are known to play an essential role in the regulation of the cell cycle (CDK1-CDK4 and CDK6), and also activate the cell cycle transcriptions (CDK9). Recently, Raj Kumar et al. published a review article which mainly dealt with the characteristics of each class of CDKs, their inhibitors, and their available X-ray crystal structures till date (Kalra et al. 2017). Several natural pyrimidines (Meridianins, Variolin B), and drugs (Imatinib, Dasatinib), were characterized as inhibitors of CDKs. CDK6 is considered as a highly validated anticancer drug target due to its essential role in regulating cell cycle

progression at G1 restriction point. The molecular docking methodology can provide a greater understanding of the ligand–protein interactions. With this motive, all the synthesized compounds were docked into the active site of enzyme.

Generally, CDK6 protein interacts with the inhibitors via hydrogen bonding (VAL101, ASP163) and hydrophobic interactions (LEU152, ILE19). According to the authors of the model, the most important for binding is the central 2-aminopyrimidine structural motif of known inhibitors. This motif enters into non-covalent interactions with amino acid residues (GLU99, VAL101) in the HIS100, which occurs only in CDK4/6 and determines the selectivity of inhibitors. Second, hydrogen bond between the ligands and the side chain of ASP104 and ASP163 also seems to contribute to a potent and selective CDK4/6 profile (Lu and Schulze-Gahmen 2006). It should be noted that due to the form of the binding site, all new compounds **2**–**21** fall into a fairly compact molecular volume (Fig. S3, Suppl. Part). This is achieved due to the presence of several flat aromatic rings that configure all the molecules in the plane of the narrow receptor cavity. Compounds **9** and **19** show different configurations coordinates (Fig. 2). It is characteristic that both compounds **9** and **19** can interact with ILE 19, forming an electrostatic interaction with anthranilate (**9**) or pyrimidine cycle (**19**). Compound **9** is capable of forming hydrogen bonds with Glu99 due to the substituent (aminogroup) in the position of C-2 pyrimidine skeleton. Compounds **9** can interact with the anion ASP163, forming an electrostatic interaction due to the π – system of the aromatic substituent in the 6 position. This amino acid residue forms a hydrogen bond with the amide group of the inhibitor Ribociclib. The phenyl ring in the 2 position of the pyrimidine core of compound **19** provides the participation of molecules in the formation of intereaction with ASP-104, as well as Ribociclib. According to the 2D interaction, some of these residues are critical structures for the CDK6 inhibition and selectivity (Kalra et al. 2017).

Inhibition of CDK9 might result in the destruction of cancer cells. This is due to CDK9 being responsible for the synthesis of Mcl-1 and XIAP antiapoptotic proteins that maintain necessary conditions for cancer cells to survive, e.g., in chronic lymphocytic leukemia or in breast cancer (Sonawane et al. 2016). Generally, the CDK9 inhibitors bind to the CDK9 protein via hydrogen bonding interactions with CYS106, PHE103 and hydrophobic interactions with ILE25, LEU156, ASP167, ALA46, LYS48 (Kalra et al. 2017, Sonawane et al. 2016). According to authors of binding models of known inhibitors and CDK9, the ATP binding site is located between the ALA46 and LEU156 conservative folded domain. All known ATP-competitive CDK9 inhibitors form hydrogen bonds with the loop portion of the binding site, simulating the location of donors and acceptors of the purine ATP cycle. The bonds are formed with oxygen of the main chain ASP104 and nitrogen of the main chain CYS106. Another feature of the binding of known inhibitors and the binding site of ATP CDK9 is the formation of a hydrogen bond with the carbonyl group ILE25, which may determine the specificity of CDK9 inhibitors. After implementing the Induced Fit Protocol, new compounds **18**, **20**, **21** demonstrated the formation of non-covalent interactions with key amino acid residues (Fig. 3).

Most frequently the new 2,4,6-trisubstituted pyrimidines 18, 20, and 21 are coordinated in the binding site by non-covalent interactions with amino acid residues ILE25 and CYS106. The formation of hydrogen bonds with LYS48 and PHE168 was also observed. Compound 20 is characterized by electrostatic interaction of the aromatic ring in the 6 position with GLU66. The pyrimidine ring of compounds 18 and 20 forms interactions of the π-systems with PHE103. Based on the data obtained, it can be concluded that the novel pyrimidines containing an additional styryl and antranilate substituent in positions C-6 and C-4, respectively, could be characterized as new scaffolds for to develop of CDK9 inhibitors.

Conclusion

In summary, a practical method for the synthesis of a novel group of 2,4,6-trisubstituted pyrimidines by the one-pot reaction of methyl 2-(*N*-acetylamino)-5-ethynylbenzoate with aroyl or *trans*-cinnamoyl chlorides and amidines has been presented. The cytotoxic activity against several cancer lines of the resulting compounds in the conventional MTT assay have been determined. The cytotoxicity data of compounds 17–21 demonstrate that they exhibit anticancer activity in micromolar range and the structural modification of pyrimidine with the introduction of a methylanthranilate moiety in the 4 position and (*E*)-styryl moiety in the 6 position or a pyrimidine fragment in the 2 position proved of great importance to obtain cytotoxic anti-cancer agents. Taken together, the present study provides a good starting point for the development of novel anti-cancer agents in the pyrimidine series as CDK9 inhibitors. Further structural optimization is in process.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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