



Design, synthesis, and antihypertensive activity of new pyrimidine derivatives endowing new pharmacophores

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Abstract

A new series of achiral pyrimidine derivatives based on nifedipine-like structure was designed and synthesized. These pyrimidyl derivatives contained hydrazine, hydrazones, acetohydrazide, differently substituted benzylidene functionalities, benzosulfohydrazine, various heterocycles such as pyrazole, pyrazolidinedione, thiazoline, and thiazolidinone rings, and fused ring systems such as triazolopyrimidine and pyrimidotriazine rings. Compounds **5a**, **5b**, **11b**, **8b**, **9b–d**, and **15b** showed a decrease in mean arterial rabbit blood pressure (MABP) ranging from 51.4 to 78.2 mmHg in rabbits in comparison with nifedipine-treated rabbits. Among these derivatives, compounds **5a**, **5b**, **9b**, and **9c** were found to exhibit calcium channel blockade activity on preparations of rabbit aortae. They exhibited relaxation in the range of 89.2% to 74.4% in comparison to nifedipine (57.6%) as well as a decrease in heart rate. Histopathological effect of compounds **5a,b** on the expression of endothelial nitric oxide synthase (eNOS) was also examined on rat aorta. An intense expression of eNOS immune staining in aortic endothelium was seen for compound **5b** indicating that it lowered blood pressure via activation of eNOS expression in aorta.

Keywords Nifedipine · Pyrimidines · ENOS · Antihypertensive · Histopathology · SAR

Introduction

Cardiovascular diseases (CVDs), including coronary heart diseases, hypertension, and peripheral artery diseases are the main cause of worldwide mortality (World Stroke

Organization 2011). Hypertension affects billions of people all around the world despite the great effort and progress in developing new drugs targeting hypertension (European Society of Hypertension 2013). Of the several classes of antihypertensive agents that have found their way in clinical use, calcium channel blockers (CCBs) have received increasing attention in recent years. They are highly recommended and are widely used as the first line anti-hypertensive therapy with few contraindications in prevention and treatment of CVDs (Lopez et al. 2006; Ozawa et al. 2006; De la Sierra and Ruilope 2007). To this day, 1,4-dihydropyridines (DHPs) are still the most potent group of CCBs with nifedipine (Adalat[®]) (Vater et al. 1972) (Fig. 1) being the initial drug treatment alone or in combination with other classes in arterial hypertension. Nifedipine, the prototype DHP, since its introduction into clinical medicine in 1975, has been modified to numerous second, third, and fourth generation commercial products for improving duration of activity, potency, and safety profiles (Ozawa et al. 2006; Yousef et al. 2005; Catterall et al. 2003; Bossert and Vater 1989). Further structure modification of the DHP core focused on the aza-analogs of DHPs resulting in the introduction of the potent dihydropyrimidine (DHPM)

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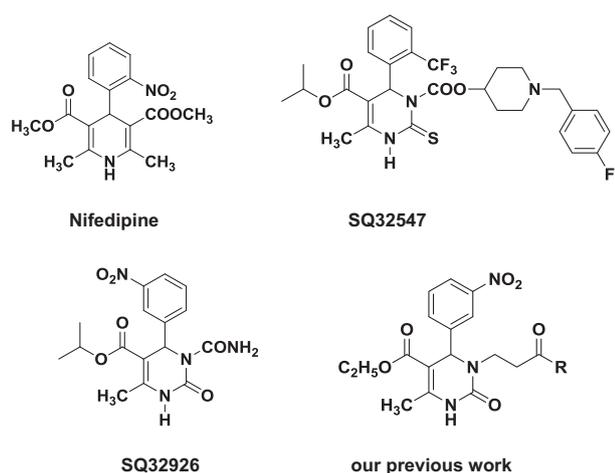


Fig. 1 Nifedipine and lead aza analogs of DHPs

derivatives, which show a very similar pharmacological profile to classical DHPs antihypertensive modulators (Kappe 1998; Grover et al. 1995; Rovnyak et al. 1995). These inherently asymmetric DHPM derivatives are not only very potent antihypertensive modulators, but have also been studied extensively to expand the existing structure–activity relationship (SAR) and to get further insight into molecular interactions at the receptor level (Kappe 1998; Grover et al. 1995; Rovnyak et al. 1995; De Fatima et al. 2015). In general, conformational features previously reported for DHP calcium channel modulators (Bikker and Weaver 1993; Gaudio et al. 1994; Palmer and Andersen 1996) were also preserved for DHPMs. The most notable difference between DHPs and DHPMs, however, is the extreme flattening of the boat-type DHPM ring around N^1 as a result of amide type bonds present in the pyrimidine core nucleus. Over the past years, several lead DHPMs have been developed among which SQ 32926 and SQ 32547 (Horovitz 1990; Negwer 1994) (Fig. 1) have been currently identified to possess antihypertensive properties. They were effective orally and found to be superior in potency and duration of antihypertensive activity to classical DHP drugs (Bikker and Weaver 1993; Kappe et al. 1997; Jain et al. 2008).

Our attention was focused on numerous pyrimidine and dihydropyrimidine derivatives bearing electron donating groups on the C4 phenyl ring that were developed and tested for biological activity (Alam et al. 2010; Kaur and Kaur 2013; Enseleit et al. 2010; Jeanneau et al. 1992) and some of them have revealed calcium channel blockade activity.

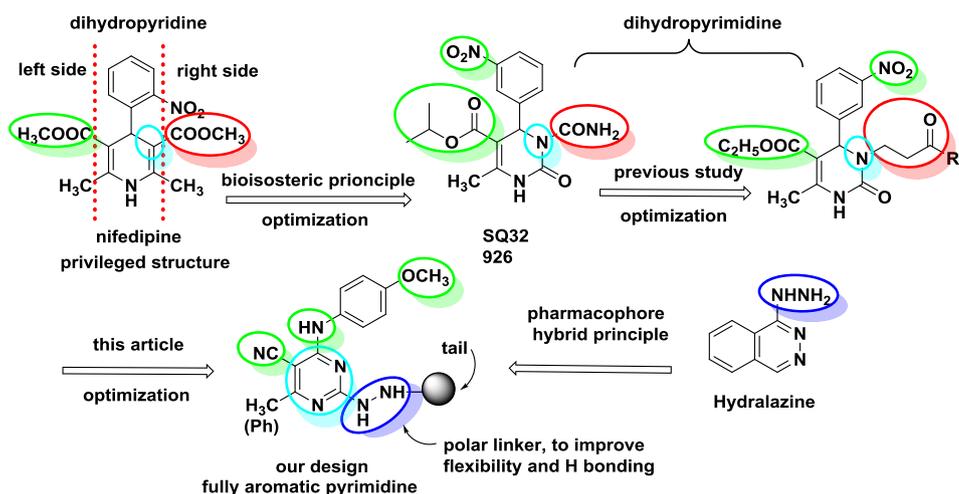
In our lab, we have explored N -containing pyrimidine scaffold and their activity profiles and we have reported, recently, a series of DHPM hybrids (Teleb et al. 2017a, b) (Fig. 1) as CCB for treatment of hypertension. Compounds

of interest were derivatized at N^3 and were chosen to comprise various functionalities as potent and selective candidates to investigate the effect of structural variations on activity modulation (Teleb et al. 2017a, b) (Fig. 1). Based on their biological results, these moieties revealed potent (Teleb et al. 2017a, b) and selective activity against hypertension.

As an extension of our previous investigations and with the aim to generate new antihypertensive agents with desirable potency and drug-like properties, our interest was focused on novel fully aromatized pyrimidine derivatives. They were designed via replacing the pyridine core present in nifedipine by the bioisosteric pyrimidine which possesses significant and privileged bioactivity and pharmacokinetic properties. As shown in Fig. 2, pyrimidine moiety is favorably used as anchor points and served as a critical moiety responsible for H-bond interactions. According to bioisosterism and molecular hybridization (Fig. 2), substituted pyrimidine, taken as lead compound, was modified at position 2 assuming that derivatization at that position of the pyrimidine backbone does not affect the left-hand side of the molecule essential for activity. This may give rise to fruitful potentially active compounds that would be of great interest. Furthermore, substitution at C2 of the pyrimidine ring system would prevent its inactivation by hydroxylation and conjugation reactions (138–141). Therefore, it was believed that pyrimidine derivatives bearing hydrazine group at C2 would provide a valuable matrix for pyrimidine functionalized calcium channel antagonism scaffold with potential application in treatment of hypertension.

Molecular design of the compounds based on pyrimidine scaffold consisted principally of 4 pharmacophores unchanged in all synthesized compounds: 1—Pyrimidine ring essential for coordination at the receptor level. 2—Phenyl ring usually substituted with an electron donating group as OCH_3 connected to non-chiral C4 pyrimidine by one atom length NH linker. 3—Additional lipophilic group at C6 position either methyl or phenyl ring and a CN moiety introduced at C5 instead of the traditional ester functional group while retaining its electron withdrawing properties trying to provide a strong H-bonding site. Modification at C4 by substituting the aromatic p -substituted aniline group for o -nitrophenyl moiety and hydrophobic methyl/phenyl groups at C6 could be of further effect to optimize the inhibitory potency. The phenyl ring could enable the molecule to make additional π – π interaction with aromatic or heteroaromatic amino acids residues in receptor binding site. 4—Additional hydrophilic group at C2, particularly $-NHNH_2$ group, substituted or unsubstituted, is a biologically active pharmacophore that has been a part of clinically used drugs as hydralazine (Ellershaw and Gurney 2001). In order to evaluate the necessity of heterocyclic

Fig. 2 Rationale for the designed compounds: bioisosterism and molecular pharmacophore hybridization



Tail = pharmacophores including hydrophobic, H-bond acceptor/donor points, heterocycles attached or fused to pyrimidine nucleus

substitution for activity, we included several fused or attached moieties as a part of the tail to establish new SAR regarding the type and size of those moieties. In the target structures, the active -NHNH_2 group was retained along with various functionalities at NH at the right hand side of the pyrimidine core and with either H-bond donor or acceptor properties to examine the influence of different size, polarity, electronic properties, flexibility, and conformational freedom of molecules on the inhibitory activity of the target molecules. Moreover, for further assessment of activity, we adopted a conformation restricted approach by adopting the strategy to link or fuse other heterocyclic moieties with the pyrimidine template in search of new lead molecules as potential drug candidates. Heterocyclic portions of these compounds could act as versatile building block to introduce different new functional groups, to anchor these rings into optimal space for binding, and may imply an important role in antihypertensive activity. Therefore, it was our intention to synthesize ring systems as pyrazole, pyrazolidinedione, thiazoline, and thiazolidinone rings in addition to fused ring systems as triazolopyrimidine and pyrimidotriazine rings to study the effect of increasing bulkiness, planarity, and polarity of such fused ring systems on their calcium antagonistic effects and specificity.

The principal features defining SARs in the present study are represented in Fig. 3.

Herein, we report the synthesis of rationally designed fully aromatic pyrimidine multifunctional derivatives. Antihypertensive evaluation and preliminary SAR study of these compounds are also described. Newly designed compounds were synthesized straightforward as depicted in Schemes 1–3.

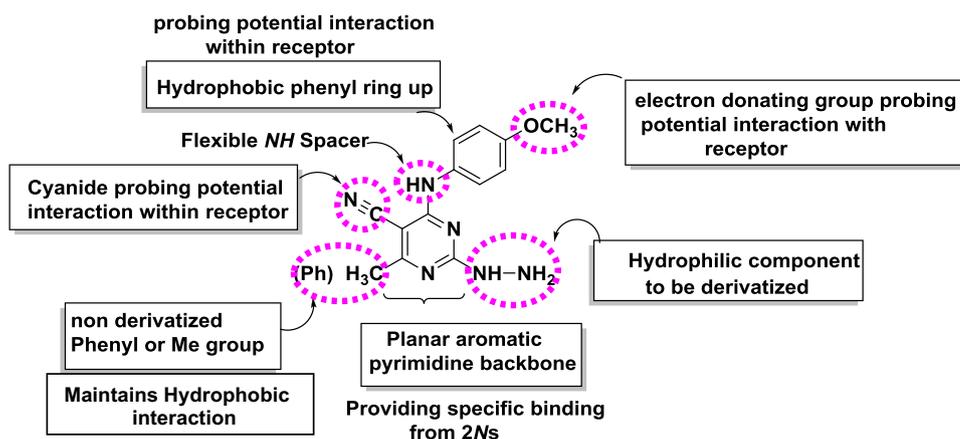
Materials and methods

Chemistry

Experimental

All melting points were uncorrected and were determined in open-glass capillaries using an electrothermal melting point apparatus (Stuart Scientific, Model SMP1, UK). IR spectra were recorded (KBr) on a Shimadzu Infrared Spectrophotometer IR 470 at the Faculty of Pharmacy, Assiut University. Nuclear magnetic resonance spectra, ^1H NMR and ^{13}C NMR, were taken using Bruker Avance III apparatus 400 MHz; Central Laboratory Unit, Faculty of Pharmacy, Cairo and Bruker DRX 400 MHz, Central Laboratory Unit, Beni-suef Universities for ^1H and 101 MHz for ^{13}C NMR, Varian EM-360L NMR spectrophotometer 60 MHz, Varian, USA, Faculty of Pharmacy, Assiut University, Varian Mercury VX 300 BB Spectrophotometer; Faculty of Science, Cairo University 300 MHz for ^1H and 75.45 MHz for ^{13}C NMR, Bruker apparatus DRX 400 MHz; Dakota State University, Brookings, SD, USA for ^1H and 101 MHz for ^{13}C NMR. TMS was used as an internal reference and chemical shifts are expressed in δ units (ppm). Mass spectra (MS) were run on a gas chromatograph/mass spectrometer Shimadzu GCMS-Qp2010 plus, single quad (70 eV), Regional Centre for Mycology and Biotechnology, Al-Azhar University, Nasr City, Cairo, and ThermoFinnigan MAT 95 XL, LR and HR work, EI, electrospray type: high resolution double-focusing magnetic sector; ESI: ESI II spray head LC/MS ThermoFinnigan LCQ Advantage Ion Trap, LR electrospray type: Ion Trap, University of South

Fig. 3 Chemical target of the present research work



Dakota, USA. All the analytical data were obtained from Microanalytical Data Unit at Cairo University, Cairo, Egypt and all results were in an acceptable range.

General method for the synthesis of 2-(methylthio)-4-methyl/phenyl-6-oxo-1,6-dihydropyrimidine-5-carbonitriles (**2a,b**)

A mixture of ethyl 2-cyano-3-phenylacrylate (**1a**) (Hussein et al. 1985) or ethyl 2-cyano-3-ethoxybutenoate (**1b**) (Yang et al. 2012) (10 mmol), *S*-methylisothiourea sulfate (11.0 mmol), and anhydrous sodium acetate (2.0 g, 24.4 mmol) in DMF (1 ml) was heated at 70 °C for 1 h. The reaction mixture was poured onto ice-cold H₂O and the formed precipitate was filtered and crystallized from EtOH (Hussein et al. 1985; Abdel-Aziz et al. 2011).

2-(Methylthio)-4-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (2a) Yield 40%; mp 140–142 °C; IR ν/cm^{-1} : 3427 (NH), 2227 (C≡N), 1557 (C=N), 1495 (C=C–Ar); ¹H-NMR (DMSO-*d*₆, 400 MHz) δ/ppm : 8.31 (s, *br*, 1H, NH), 2.25 (s, 3H, S-CH₃), 2.11 (s, 3H, CH₃) (Hussein et al. 1985).

2-(Methylthio)-4-phenyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (2b) Yield 60%; mp 276–277 °C; IR ν/cm^{-1} : 3412 (NH), 2228 (C≡N), 1593 (C=N), 1573 (C=C–Ar) (Hussein et al. 1985; Abdel-Aziz et al. 2011).

General method for the synthesis of 4-chloro-6-methyl/phenyl-2-(methylthio)-pyrimidine-5-carbonitriles (**3a,b**)

A mixture of **2a,b** (1.49 mmol), POCl₃ (0.4 ml, 4 mmol) and *N,N*-dimethylaniline (0.2 ml, 1.58 mmol) was heated at 60 °C for 2 h. After completion of the reaction as indicated by TLC, the mixture was poured onto ice-cold H₂O and the formed precipitate was filtered and crystallized from EtOH (Vasudevan et al. 2012, 2014).

4-Chloro-6-methyl-2-(methylthio)-pyrimidine-5-carbonitrile (3a) Yield 93%; mp 88–90 °C; IR ν/cm^{-1} : 3300 (NH), 2215 (C≡N), 1614 (C=N), 1604 (C=C–Ar), 1263, 1134 (ν_{as} and ν_{s} C–O–C).

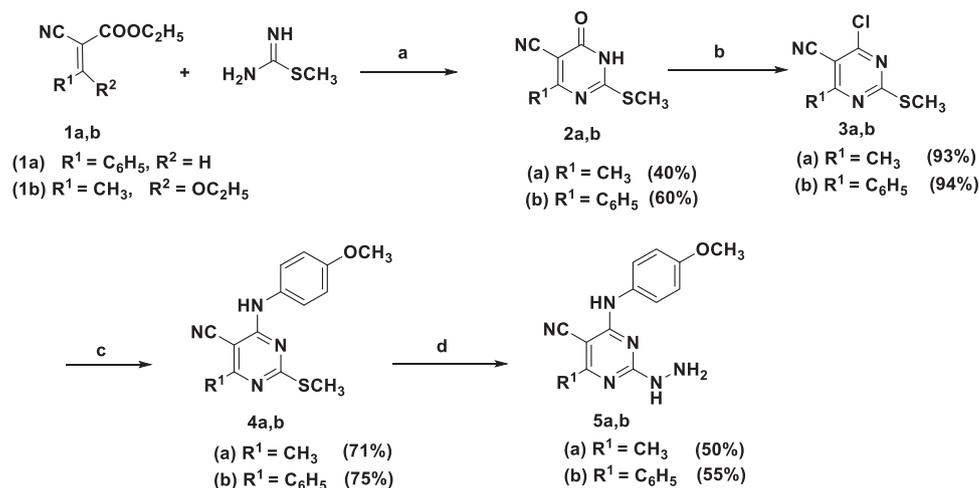
4-Chloro-6-phenyl-2-(methylthio)-pyrimidine-5-carbonitrile (3b) Yield 94%; mp 141–140 °C; IR ν/cm^{-1} : 3312 (NH), 2214 (C≡N), 1614 (C=N), 1572 (C=C–Ar), 1293, 1089 (ν_{as} and ν_{s} C–O–C).

General method for the synthesis of 4-(4-methoxyanilino)-6-methyl/phenyl-2-(methylthio)-pyrimidine-5-carbonitriles (**4a,b**)

A mixture of **3a,b** (2.86 mmol), 4-methoxyaniline (0.36 g, 2.88 mmol) and anhydrous K₂CO₃ (0.4 g, 5.76 mmol) in EtOH (15 ml) was heated under reflux for 5 h. It was then cooled, filtered and the filtrate was concentrated to half its volume. The obtained precipitate was filtered and crystallized from EtOH-H₂O (3:1 v/v).

4-(4-Methoxyanilino)-6-methyl-2-(methylthio)-pyrimidine-5-carbonitrile (4a) Yield 71%; mp 158–160 °C; IR ν/cm^{-1} : 3300 (NH), 2214 (C≡N), 1614 (C=N), 1604 (C=C–Ar), 1293, 1134 (ν_{as} and ν_{s} C–O–C); ¹H NMR (CDCl₃, 60 MHz) δ/ppm : 7.49 (d, 2H, Ar-Hs, *o*- to NH-Ar, *J* = 12.6 Hz), 7.21 (s, *br*, 1H, NH), 6.90 (d, 2H, Ar-Hs, *o*- to OCH₃, *J* = 12.0 Hz), 3.83 (s, 3H, OCH₃), 2.58 (s, 3H, S-CH₃), 2.50 (s, 3H, CH₃); EIMS *m/z* 286 (M⁺, 100), 271 (33), 270 (25), 238 (13); Anal. Calcd. for C₁₄H₁₄N₄OS: (286.35): C, 58.72; H, 4.93; N, 19.72. Found: C, 58.97; H, 4.98; N, 19.57.

4-(4-Methoxyanilino)-6-phenyl-2-(methylthio)-pyrimidine-5-carbonitrile (4b) Yield 75%; mp 190–192 °C; IR ν/cm^{-1} : 3312 (NH), 2215 (C≡N), 1622 (C=N), 1572 (C=C–Ar), 1263, 1089 (ν_{as} and ν_{s} C–O–C); ¹H NMR (CDCl₃, 400 MHz) δ/ppm : 7.97 (d, 2H, C_{2,6}-Hs of phenyl ring, *J* = 8.0

Scheme 1 Synthetic pathway to hydrazine derivatives **5a,b**

Hz), 7.46–7.55 (m, 4H, C3,4,5-*Hs* of phenyl ring, + *NH*-Ar), 7.25 (d, 2H, Ar-*Hs*, *o*- to *NH*-Ar, $J = 6.0$ Hz), 6.91 (d, 2H, Ar-*Hs*, *o*- to OCH_3 , $J = 9.2$ Hz), 3.82 (s, 3H, OCH_3), 2.50 (s, 3H, *S*- CH_3); Anal. Calcd. for $\text{C}_{19}\text{H}_{16}\text{N}_4\text{OS}$ (348.42): C, 65.50; H, 4.93; N, 16.08. Found: C, 65.78; H, 4.98; N, 16.34.

General method for the synthesis of 2-hydrazinyl-6-methyl/phenyl-4-(4-methoxyanilino)-pyrimidine-5-carbonitriles (**5a,b**)

A mixture of **4a,b** (0.24 mmol), and hydrazine hydrate (0.012 g, 0.24 mmol) in dioxane (1 ml) was heated under reflux for 4 h. The reaction mixture was concentrated to half its volume and the obtained precipitate was filtered and crystallized from EtOH.

2-Hydrazinyl-6-methyl-4-(4-methoxyanilino)-pyrimidine-5-carbonitrile (5a) Yield 50%; mp 190–192 °C; IR ν/cm^{-1} : 3326 (NH), 3215 (NH + NH_2), 2188 ($\text{C}\equiv\text{N}$), 1610 ($\text{C}=\text{N}$), 1490 ($\text{C}=\text{C}$ -Ar), 1180, 1094 (ν_{as} and ν_{s} C–O–C); ^1H NMR (DMSO- d_6 , 60 MHz) δ/ppm : 8.60 (s, *br*, 1H, C2-*NH*), 8.35 (s, *br*, 1H, *NH*-Ar), 7.63 (d, 2H, Ar-*Hs*, *o*- to *NH*-Ar, $J = 11.4$ Hz), 6.91 (d, 2H, Ar-*Hs*, *o*- to OCH_3 , $J = 11.4$ Hz), 3.88 (s, 3H, OCH_3), 2.44 (s, 3H, CH_3); Anal. Calcd. for $\text{C}_{13}\text{H}_{14}\text{N}_6\text{O}$ (270.29): C, 57.77; H, 5.22; N, 31.09. Found: C, 57.96; H, 5.34; N, 31.36.

2-Hydrazinyl-6-phenyl-4-(4-methoxyanilino)-pyrimidine-5-carbonitrile (5b) Yield 55%; mp 215–217 °C; IR ν/cm^{-1} : 3287 (NH), 3270 (NH + NH_2), 2188 ($\text{C}\equiv\text{N}$), 1612 ($\text{C}=\text{N}$), 1582 ($\text{C}=\text{C}$ -Ar), 1251, 1032 (ν_{as} and ν_{s} C–O–C); ^1H NMR (DMSO- d_6 , 400 MHz) δ/ppm : 9.12 (s, *br*, 1H, C2-*NH*, D_2O exchangeable), 8.96 (s, *br*, 1H, *NH*-Ar, D_2O exchangeable), 7.53–7.90 (m, 7H, Ar-*Hs*), 6.90 (d, 2H, Ar-*Hs*, *o*- to OCH_3 , $J = 8.92$ Hz), 4.38 (s, 2H, NH_2 , D_2O exchangeable), 3.76 (s, 3H, OCH_3); Anal. Calcd. for $\text{C}_{18}\text{H}_{16}\text{N}_6\text{O}$ (332.36): C,

65.05; H, 4.85; N, 25.29. Found: C, 65.17; H, 4.91; N, 25.43.

General method for the synthesis of 8-(4-Methoxyanilino)-6-methyl-3-aryl-4*H*-pyrimido[2,1-*c*][1,2,4]-triazine-7-carbonitriles (**6a–c**)

The appropriately substituted phenacyl bromide (1 mmol) was added to a solution of the hydrazine derivative **5a** (0.27 g, 1 mmol) in absolute EtOH (5 ml). The reaction mixture was heated under reflux for 2 h, concentrated to half its volume then left to cool to RT. The obtained precipitate was filtered, washed with petroleum ether (40°–60°), dried and crystallized from EtOH.

8-(4-Methoxyanilino)-6-methyl-3-(4-bromophenyl)-4*H*-pyrimido[2,1-*c*][1,2,4]-triazine-7-carbonitrile (6a) Yield 60%; mp 200–203 °C; IR ν/cm^{-1} : 3565 (NH), 2223 ($\text{C}\equiv\text{N}$), 1617 ($\text{C}=\text{N}$), 1576 ($\text{C}=\text{C}$ -Ar), 1254, 1026 (ν_{as} and ν_{s} C–O–C); ^1H NMR (DMSO- d_6 , 400 MHz) δ/ppm : 12.63 (s, 1H, *NH*-triazine), 10.77 (s, 1H, *NH*-Ar), 7.83 (d, 2H, Ar-*Hs*, *o*- to Br, $J = 8.68$ Hz), 7.75 (d, 2H, Ar-*Hs*, *m*- to Br, $J = 8.68$ Hz), 7.44 (d, 2H, Ar-*Hs*, *o*- to *NH*-Ar, $J = 8.72$ Hz), 7.02 (d, 2H, Ar-*Hs*, *o*- to OCH_3 , $J = 9.0$ Hz), 5.32 (s, 2H, C4-*H*), 3.80 (s, 3H, OCH_3), 2.82 (s, 3H, CH_3); ^{13}C NMR (DMSO- d_6 , 101 MHz) δ/ppm : 168.6 (CN), 160.0 (C-4 of 4-bromophenyl), 159.2 (C-7), 159.0 (C-9a), 148.7 (C-6, C-1 of 4-methoxyanilino), 145.1 (C-8), 134.2 (C-3, C-5 of 4-bromophenyl), 132.8 (C-3), 130.0 (C-4 of 4-methoxyanilino), 129.3 (C-2, C-6 of 4-methoxyanilino), 126.4 (C-2, C-6 of 4-bromophenyl), 124.8 (C-1 of 4-bromophenyl), 114.0 (C-3, C-5 of 4-methoxyanilino), 55.39 (OCH_3), 44.9 (C-4), 18.9 (C6- CH_3); HRESIMS $\text{M}^+ + 1$ at m/z 449.0729 (100) and $\text{M}^+ + 3$ at m/z 451.0712 (94) corresponding to $\text{C}_{21}\text{H}_{17}^{79}\text{BrN}_6\text{O}$ and $\text{C}_{21}\text{H}_{17}^{81}\text{BrN}_6\text{O}$ radical cation fragments; calculated = 448 (450); Anal. Calcd for

$C_{21}H_{17}BrN_6O$ (449.30): C, 56.14; H, 3.81; N, 18.70. Found: C, 56.30; H, 3.78; N, 18.86.

8-(4-Methoxyanilino)-6-methyl-3-(4-chlorophenyl)-4H-pyrimido[2,1-c][1,2,4]-triazine-7-carbonitrile (6b) Yield 52%; mp 193–195 °C; IR ν/cm^{-1} : 3399 (NH), 2223 (C≡N), 1624 (C=N), 1509 (C=C–Ar), 1232, 1090 (ν_{as} and ν_s C–O–C); 1H NMR (DMSO- d_6 , 400 MHz) δ ppm: 12.64 (s, 1H, NH-triazine), 10.78 (s, 1H, NH-Ar), 7.91 (d, 2H, Ar-Hs, *o*- to Cl, $J = 7.6$ Hz), 7.60 (d, 2H, Ar-Hs, *m*- to Cl, $J = 8.0$ Hz), 7.45 (d, 2H, Ar-Hs, *o*- to NH-Ar, $J = 8.0$ Hz), 7.02 (d, 2H, Ar-Hs, *o*- to OCH₃, $J = 8.4$ Hz), 5.34 (s, 2H, C4-H₂), 3.79 (s, 3H, OCH₃), 2.83 (s, 3H, CH₃); Anal. Calcd. for $C_{21}H_{17}ClN_6O$ (404.85): C, 62.30; H, 4.23; N, 20.76. Found: C, 62.57; H, 4.30; N, 20.92.

8-(4-Methoxyanilino)-6-methyl-3-(4-nitrophenyl)-4H-pyrimido[2,1-c][1,2,4]-triazine-7-carbonitrile (6c) Yield 65%; mp 205–207 °C; IR ν/cm^{-1} : 3565 (NH), 2230 (C≡N), 1635 (C=N), 1507 (C=C–Ar), 1457, 1346 (ν_{as} and ν_s NO₂), 1254, 1032 (ν_{as} and ν_s C–O–C); 1H NMR (DMSO- d_6 , 400 MHz) δ /ppm: 12.80 (s, *br*, 1H, NH-triazine, D₂O exchangeable), 10.85 (s, *br*, 1H, NH-Ar, D₂O exchangeable), 8.36 (d, 2H, Ar-Hs, *o*- to NO₂, $J = 8.84$ Hz), 8.14 (d, 2H, Ar-Hs, *m*- to NO₂, $J = 8.8$ Hz), 7.44 (d, 2H, Ar-Hs, *o*- to NH-Ar, $J = 8.68$ Hz), 7.03 (d, 2H, Ar-Hs, *o*- to OCH₃, $J = 8.88$ Hz), 5.38 (s, 2H, C4-H₂), 3.80 (s, 3H, OCH₃), 2.83 (s, 3H, CH₃); Anal. Calcd. for $C_{21}H_{17}N_7O_3$ (415.14): C, 60.72; H, 4.12; N, 23.60. Found: C, 60.89; H, 4.14; N, 23.84.

General method for the synthesis of 7-(4-methoxyanilino)-3,5-disubstituted-[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitriles (7a–f)

For **7a,b,d,e**: A solution of the hydrazine derivative **5a,b** (1 mmol) and the appropriate aliphatic acid (5 ml) was heated under reflux for 7 h. The reaction mixture was concentrated to a small volume and diluted with ice-cold H₂O. The obtained precipitate was filtered, washed with H₂O, dried and crystallized from EtOH/H₂O.

For **7c,f**: A solution of the hydrazine derivative **5a,b** (1 mmol), benzoic acid (0.122 g, 1 mmol) and POCl₃ (0.4 ml) was heated under reflux for 4 h. After cooling, the reaction mixture was poured onto ice-cold H₂O and neutralized with a saturated solution of NaHCO₃. The precipitate was collected by filtration, washed with cold H₂O and recrystallized from EtOH.

7-(4-Methoxyanilino)-5-methyl-[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile (7a) Yield 23%; mp 178–180 °C; IR ν/cm^{-1} : 3213 (NH), 2202 (C≡N), 1626 (C=N), 1581 (C=C–Ar), 1231, 1033 (ν_{as} and ν_s C–O–C); 1H NMR (DMSO- d_6 ,

300 MHz) δ /ppm: 9.87 (s, *br*, 1H, NH-Ar, D₂O-exchangeable), 9.50 (d, 1H, triazole NH, $J = 10.5$ Hz, D₂O-exchangeable), 9.21 (s, *br*, 1H, pyrimidine N-H, D₂O-exchangeable), 8.02 (s, 1H, C3-H), 7.50 (d, 2H, Ar-Hs, *o*- to NH-Ar, $J = 6.9$ Hz), 6.87 (d, 2H, Ar-Hs, *o*- to OCH₃, $J = 6.9$ Hz), 3.74 (s, 3H, OCH₃), 2.37 (s, 3H, CH₃); ^{13}C NMR (DMSO- d_6 , 75 MHz) δ /ppm: 171.7 (CN), 167.2 (C-6), 161.5 (C-5), 160.3 (C-9), 159.8 (C-7), 131.0 (C-1 of 4-methoxyaniline), 125.2 (C-3), 123.9 (C-4 of 4-methoxyaniline), 113.5, 113.1 (C-5,C-3 of 4-methoxyaniline), 116.2, 116.1 (C-6, C-2 of 4-methoxyaniline), 55.1 (OCH₃), 23.0 (C-5-CH₃); Anal. Calcd. for $C_{14}H_{12}N_6O$ (280.28): C, 59.99; H, 4.32; N, 29.98. Found: C, 60.14; H, 4.39; N, 30.16.

7-(4-Methoxyanilino)-3,5-dimethyl-[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile (7b) Yield 32%; mp 185–187 °C; IR ν/cm^{-1} : 3315 (NH), 2216 (C≡N), 1626 (C=N), 1577 (C=C–Ar), 1254, 1022 (ν_{as} and ν_s C–O–C); 1H NMR (DMSO- d_6 , 400 MHz) δ /ppm: 9.78 (s, *br*, 1H, NH-Ar, D₂O-exchangeable), 9.28 (s, 1H, triazole N-H), 9.15 (s, 1H, pyrimidine N-H, D₂O-exchangeable), 7.50 (d, 2H, Ar-Hs, *o*- to NH-Ar, $J = 8.8$ Hz), 6.85 (d, 2H, Ar-Hs, *o*- to OCH₃, $J = 8.4$ Hz), 3.74 (s, 3H, OCH₃), 2.36 (s, 3H, C5-CH₃), 1.85 (s, 3H, C3-CH₃); Anal. Calcd. for $C_{15}H_{14}N_6O$ (294.12): C, 61.21; H, 4.79; N, 28.55. Found: C, 61.42; H, 4.87; N, 28.17.

7-(4-Methoxyanilino)-3-phenyl-5-methyl-[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile (7c) Yield 40%; mp 203–202 °C; IR ν/cm^{-1} : 3340 (NH), 2212 (C≡N), 16211 (C=N), 1585 (C=C–Ar), 1249, 1042 (ν_{as} and ν_s C–O–C); 1H NMR (DMSO- d_6 , 400 MHz) δ /ppm: 10.84 (s, 1H, NH-Ar, D₂O-exchangeable), 8.25 (*dd*, 2H, C2,6-Hs of C3-phenyl, $J_{AM} = 7.9$ Hz, $J_{AX} = 2.3$ Hz), 7.56–7.61 (m, 3H, C3,4,5-Hs of C3-phenyl), 7.41 (d, 2H, Ar-Hs, *o*- to NH-Ar, $J = 8.84$ Hz), 7.02 (d, 2H, Ar-Hs, *o*- to OCH₃, $J = 8.88$ Hz), 3.82 (s, 3H, OCH₃), 2.51 (s, 3H, C5-CH₃); EIMS m/z : 357 (M⁺, 25), 356 (99), 341 (70), 185 (67), 92 (32), 77 (100), 76 (31); Anal. Calcd. for $C_{20}H_{16}N_6O$ (356.14): C, 67.40; H, 4.53; N, 23.58. Found: C, 67.64; H, 4.59; N, 23.71.

7-(4-Methoxyanilino)-5-phenyl-[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile (7d) Yield 20%; mp 222–220 °C; IR ν/cm^{-1} : 3199 (NH), 2212 (C≡N), 1624 (C=N), 1583 (C=C–Ar), 1248, 1042 (ν_{as} and ν_s C–O–C); 1H NMR (DMSO- d_6 , 400 MHz) δ /ppm: 9.79 (s, *br*, 1H, NH-Ar, D₂O-exchangeable), 9.58 (d, *br*, 1H, triazole NH, $J = 10$ Hz, D₂O-exchangeable), 9.30 (s, *br*, 1H, pyrimidine NH, D₂O-exchangeable), 8.01 (s, 1H, C3-H), 7.81 (d, 2H, Ar-Hs, *o*- to NH-Ar, $J = 7.68$ Hz), 7.53–7.57 (m, 5H, Ar-Hs), 6.90 (d, *dist*, 2H, Ar-Hs, *o*- to OCH₃, $J = 7.92$ Hz), 3.76 (s, 3H, OCH₃); Anal. Calcd. for $C_{19}H_{14}N_6O$ (342.12): C, 66.66; H, 4.12; N, 24.55. Found: C, 66.81; H, 4.09; N, 24.69.

7-(4-Methoxyanilino)-3-methyl-5-phenyl-[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile (7e) Yield 40%; mp 213–215 °C; IR ν/cm^{-1} : 3270 (NH), 2208 (C \equiv N), 1618 (C=N), 1580 (C=C–Ar), 1245, 1038 (ν_{as} and ν_{s} C–O–C); ^1H NMR (DMSO- d_6 , 400 MHz) δ/ppm : 9.77 (s, *br*, 1H, *NH*-Ar), 9.50 (s, 1H, triazole *H*), 9.30 (s, 1H, triazole *N*-*H*), 7.81 (d, 2H, C2,6-*Hs* of C-3-phenyl, $J = 5.2$ Hz), 7.51–7.57 (m, 3H, C3,4,5-*Hs* of C-5-phenyl), 7.53 (d, 2H, Ar-*Hs*, *o*- to *NH*-Ar, $J = 8.8$ Hz), 6.89 (d, 2H, Ar-*Hs*, *o*- to OCH_3 , $J = 8.8$ Hz), 3.75 (s, 3H, OCH_3), 1.87 (s, 3H, CH_3); Anal. Calcd. for $\text{C}_{20}\text{H}_{16}\text{N}_6\text{O}$ (356.14): C, 67.40; H, 4.53; N, 23.58. Found: C, 67.63; H, 4.59; N, 23.74.

7-(4-Methoxyanilino)-3,5-diphenyl-[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile (7f) Yield 43%; mp 287–289 °C; IR ν/cm^{-1} : 3346 (NH), 2212 (C \equiv N), 1603 (C=N), 1573 (C=C–Ar), 1252, 1029 (ν_{as} and ν_{s} C–O–C); ^1H NMR (DMSO- d_6 , 400 MHz) δ/ppm : 11.00 (s, *br*, 1H, *NH*-Ar), 7.83, 8.76 (2d, 4H, Ar-*Hs* of C-5-phenyl, $J = 6.0$ Hz), 7.54–7.75 (m, 6H, 5 Ar-*Hs* of C-3-phenyl + 1 Ar-*H* of C-5-phenyl), 7.49 (d, 2H, Ar-*Hs*, *o*- to *NH*-Ar, $J = 8.0$ Hz), 7.03 (d, 2H, Ar-*Hs*, *o*- to OCH_3 , $J = 7.6$ Hz), 3.80 (s, 3H, OCH_3); Anal. Calcd. for $\text{C}_{25}\text{H}_{18}\text{N}_6\text{O}$ (418.15): C, 71.76; H, 4.34; N, 20.08. Found: C, 71.89; H, 4.41; N, 20.29.

General method for the synthesis of 2-(5-Amino-4-cyano-3-methyl-1H-pyrazol-1-yl)-6-methyl/phenyl-4-(4-methoxyanilino)-pyrimidine-5-carbonitriles (8a,b)

A mixture of the hydrazine derivative **5a,b** (0.5 mmol) and 2-(1-ethoxy-ethylidene)malononitrile (**1b**) (Yang et al. 2012) (0.13 g, 0.5 mmol) in absolute EtOH (5 ml) was heated under reflux for 5 h and left to cool to RT. The separated products were filtered, washed with petroleum ether, dried, and crystallized from EtOH.

2-(5-Amino-4-cyano-3-methyl-1H-pyrazol-1-yl)-6-methyl-4-(4-methoxyanilino)-pyrimidine-5-carbonitrile (8a) Yield 23%; mp 140–143 °C; IR ν/cm^{-1} : 3389 (NH), 2221 (C \equiv N), 1635 (C=N), 1598 (C=C–Ar), 1258, 1030 (ν_{as} and ν_{s} C–O–C); ^1H NMR (DMSO- d_6 , 400 MHz) δ/ppm : 10.04 (s, *br*, 1H, *NH*-Ar), 9.24 (s, *br*, 2H, pyrazole-*NH*₂), 7.83 (m, 2H, Ar-*Hs*, *o*- to *NH*-Ar), 6.94 (m, 2H, Ar-*Hs*, *o*- to OCH_3), 3.76 (s, 3H, OCH_3), 2.41 (s, 3H, C6- CH_3 -pyrimidine), 2.15 (s, 3H, C3- CH_3 -pyrazole); Anal. Calcd. for $\text{C}_{18}\text{H}_{16}\text{N}_8\text{O}$ (360.37): C, 59.99; H, 4.48; N, 31.09. Found: C, 60.18; H, 4.57; N, 31.34.

2-(5-Amino-4-cyano-3-methyl-1H-pyrazol-1-yl)-6-phenyl-4-(4-methoxyanilino)-pyrimidine-5-carbonitrile (8b) Yield 21%; mp 192–190 °C; IR ν/cm^{-1} : 3315 (NH), 2216 (C \equiv N), 1635 (C=N), 1578 (C=C–Ar), 1248, 1035 (ν_{as} and ν_{s} C–O–C); ^1H NMR (DMSO- d_6 , 400 MHz) δ/ppm : 10.15 (s, *br*,

1H, *NH*-Ar), 7.88 (d, 2H, Ar-*Hs*, $J = 6.80$ Hz), 7.79 (s, dist, 2H, pyrazole-*NH*₂), 7.55–7.79 (m, 3H, Ar-*Hs*), 7.44 (d, 2H, Ar-*Hs*, *o*- to *NH*-Ar, $J = 8.68$ Hz), 7.00 (d, 2H, Ar-*Hs*, *o*- to OCH_3 , $J = 8.72$ Hz), 3.81 (s, 3H, OCH_3), 2.16 (s, 3H, C3- CH_3 -pyrazole); EIMS m/z : 422 (M^+ , 49), 421 (36), 357 (54), 333 (22), 332 (100), 317 (33), 302 (30); Anal. Calcd. for $\text{C}_{23}\text{H}_{18}\text{N}_8\text{O}$ (422.16): C, 65.39; H, 4.29; N, 26.53. Found: C, 65.61; H, 4.32; N, 26.79.

General method for the synthesis of 2-(4-Cyano-5-oxo-3-aryl-4,5-dihydro-1H-pyrazol-1-yl)-6-methyl/phenyl-4-(4-methoxyanilino)-pyrimidine-5-carbonitriles (9a-d)

A mixture of the hydrazine derivative **5a,b** (0.5 mmol) and ethyl 2-cyano-3-phenyl acrylate **1a** or ethyl 2-cyano-3-methoxyphenylacrylate⁽¹⁴³⁾ **1b** (0.1 g, 0.5 mmol) in absolute EtOH (5 ml) was heated under reflux for 5 h and left to cool to RT. The separated products were filtered, washed with petroleum ether dried and crystallized from EtOH.

2-(4-Cyano-5-oxo-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-6-methyl-4-(4-methoxyanilino)-pyrimidine-5-carbonitrile (9a) Yield 40%; mp 170–172 °C; IR ν/cm^{-1} : 3275 (OH), 3187 (NH), 2360, 2213 (2 \times C \equiv N), 1606 (C=N), 1561 (C=C–Ar), 1233, 1028 (ν_{as} and ν_{s} C–O–C); ^1H NMR (DMSO- d_6 , 400 MHz) δ/ppm : 11.60 (s, *br*, 1H, *OH*, C5-*OH*-pyrazole), 9.23 (s, *br*, 1H, *NH*-Ar), 8.16 (s, 1H, C4-*H*-pyrazole), 7.69 (d, 2H, Ar-*Hs*, *o*- to *NH*-Ar, $J = 6.52$ Hz), 7.37–7.46 (m, 5H, Ar-*Hs* of C-3-pyrazole), 6.93 (d, 2H, Ar-*Hs*, *o*- to OCH_3 , $J = 8.72$ Hz), 3.79 (s, 3H, OCH_3), 2.43 (s, 3H, CH_3); ^{13}C NMR (DMSO- d_6 , 101 MHz) δ/ppm : 172.7 (2 \times CN), 160.9 (C5), 159.3 (C2), 156.3 (C6), 143.7 (C4), 135.4 (C-1, C-4 of 4-methoxyaniline), 132.3 (C-3, C-5-pyrazole), 129.9 (C4-pyrazole), 129.2 (C-2, C-6 of 4-methoxyaniline), 127.0 (C-3, C-5 of 4-methoxyaniline), 124.4 (C-1, C-4 of phenyl at C3-pyrazole), 117.0 (C-2, C-6 of phenyl at C3-pyrazole), 113.8 (C-3, C-5 of phenyl at C3-pyrazole), 55.7 (OCH_3), 23.6 (C6- CH_3); EIMS m/z 423 (M^+ , 10), 382 (16), 357 (22), 325 (24), 312 (35), 309 (20), 296 (23), 286 (100), 281 (28), 280 (63), 244 (85), 210 (35), 207 (38), 201 (40), 191 (37), 190 (38), 184 (30), 181 (47), 178 (24), 176 (25), 163 (63), 145 (35), 144 (95), 141 (38), 121 (60); Anal. Calcd. for $\text{C}_{23}\text{H}_{17}\text{N}_7\text{O}$ (423.43): C, 65.24; H, 4.05; N, 23.16. Found: C, 65.39; H, 4.11; N, 23.42.

2-(4-Cyano-5-oxo-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-6-methyl-4-(4-methoxyanilino)-pyrimidine-5-carbonitrile (9b) Yield 45%; mp 188–190 °C; IR ν/cm^{-1} : 3282 (OH), 3280 (NH), 2320, 2215 (2 \times C \equiv N), 1619 (C=N), 1577 (C=C–Ar), 1244, 1035 (ν_{as} and ν_{s} C–O–C); ^1H NMR (DMSO- d_6 , 400 MHz) δ/ppm : 11.60 (s, *br*, 1H, *OH*, C⁵-*OH*-pyrazole, D_2O exchangeable), 9.18 (s, *br*, 1H, *NH*-Ar, D_2O exchangeable), 8.11 (s, 1H, C4-*H*-pyrazole),

7.61–7.91 (m, 4H, Ar-Hs of 4-methoxyphenyl at C3-pyrazole), 7.00 (d, 2H, Ar-Hs, *o*- to NH-Ar, $J = 8.36$ Hz), 6.93 (d, 2H, Ar-Hs, *o*- to OCH₃, $J = 8.8$ Hz), 3.78 (s, 6H, 2 × OCH₃), 2.41 (s, 3H, CH₃); ¹³C NMR (DMSO-d₆, 101 MHz) δ/ppm: 172.0 (2 × CN), 160.9 (pyrazole-C4 + C5), 160.8 (C5), 159.2 (C2), 156.1 (C6), 143.8 (C4), 132.5 (C1 of 4-methoxyaniline), 128.5 (pyrazole-C3), 128.0 (C-1 of 4-methoxyphenyl at C3-pyrazole), 124.2 (C-4 of 4-methoxyaniline + C4 of 4-methoxyphenyl at C3-pyrazole), 117.1 (C-2, C-6 of 4-methoxyphenyl at C3-pyrazole), 114.7 (C-2, C-6 of 4-methoxyaniline), 113.8 (C-3, C-5 of 4-methoxyphenyl at C3-pyrazole + C-3, C-5 of 4-methoxyaniline), 55.8, 55.7 (2 × OCH₃), 23.6 (C-6-CH₃); EIMS *m/z*: 453 (M⁺, 2), 390 (24), 389 (100), 388 (39), 387 (35), 386 (23); Anal. Calcd. for C₂₄H₁₉N₇O₃ (453.45): C, 63.57; H, 4.22; N, 21.62. Found: C, 63.80; H, 4.27; N, 21.74.

2-(4-Cyano-5-oxo-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-6-phenyl-4-(4-methoxyanilino)-pyrimidine-5-carbonitrile (9c) Yield 41%; mp 191–193 °C; IR ν/cm^{-1} : 3400 (OH), 3305 (NH), 2222, 2200 (2 × C≡N), 1621 (C=N), 1584 (C=C-Ar), 1231, 1028 (ν_{as} and ν_{s} C–O–C); ¹H NMR (DMSO-d₆, 400 MHz) δ/ppm: 11.79 (s, *br*, 1H, OH, C5-OH-pyrazole), 9.33 (s, *br*, 1H, NH-Ar), 8.21 (s, 1H, C4-H-pyrazole), 7.45–7.96 (m, 12H, Ar-Hs, 2 × 5-phenyl-Hs + 2 Ar-Hs, *o*- to NH-Ar), 6.97 (d, 2H, Ar-Hs, *o*- to OCH₃, $J = 6.64$ Hz), 3.81 (s, 3H, OCH₃); ¹³C NMR (DMSO-d₆, 101 MHz) δ/ppm: 180.1 (2 × CN), 160.7 (C5), 159.6 (C2), 157.5 (C6), 142.0 (C4), 138.5 (C-1 of 4-methoxyaniline), 135.5 (C-4, C-5 pyrazole), 132.0 (C4 of 4-methoxyaniline), 129.8 (C-1 of phenyl at C3-pyrazole + C3-pyrazole), 129.1 (C-2, C-6 of 4-methoxyaniline), 128.5 (C-1 of phenyl at C6-pyrimidine), 128.3 (C-2, C-6 of phenyl at C6-pyrimidine), 126.5 (C-2, C-6 of phenyl at C3-pyrazole), 124.4 (C-3, C-5 of phenyl at C6-pyrimidine), 117.1 (C-3, C-5 of 4-methoxyaniline), 113.2 (C-3, C-5 of phenyl at C3-pyrazole), 83.75 (C4 of phenyl at C3-pyrazole + C4 of phenyl at C-6-pyrimidine), 55.19 (OCH₃); EIMS *m/z*: 486 (M⁺, 2), 317 (23), 316 (22), 199 (24), 175 (34), 171 (41), 158 (28), 144 (25), 141 (49), 140 (50), 131 (23), 114 (22), 111 (70), 110 (27), 109 (23), 66 (100); Anal. Calcd. for C₂₈H₁₉N₇O₂ (485.50): C, 69.27; H, 3.94; N, 20.20. Found: C, 69.67; H, 3.76; N, 20.52.

2-(4-Cyano-5-oxo-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-6-phenyl-4-(4-methoxyanilino)-pyrimidine-5-carbonitrile (9d) Yield 43%; mp 201–203 °C; IR ν/cm^{-1} : 3331 (OH), 3187 (NH), 2305, 2206 (2 × C≡N), 1608 (C=N), 1586 (C=C-Ar), 1247, 1031 (ν_{as} and ν_{s} C–O–C); ¹H NMR (DMSO-d₆, 400 MHz) δ/ppm: 11.65 (s, *br*, 1H, OH, C5-OH-pyrazole, D₂O exchangeable), 9.28 (s, *br*, 1H, NH-Ar, D₂O exchangeable), 8.15 (s, 1H, C4-H-pyrazole), 7.56–7.96 (m, 9H, Ar-Hs), 7.02 (d, 2H, Ar-Hs, *o*- to NH-Ar,

$J = 7.72$ Hz), 6.96 (d, 2H, Ar-Hs, *o*- to OCH₃, $J = 8.88$ Hz), 3.82, 3.81 (2s, 6H, 2 × OCH₃); ¹³C NMR (DMSO-d₆, 101 MHz) δ/ppm: 172.8, 169.5 (2 × CN), 161.6 (C4-pyrazole), 160.6 (C5), 156.5 (C2), 153.1 (C6), 148.1 (C4), 131.4 (C5-pyrazole), 129.8 (C3-pyrazole), 128.5 (C1 of 4-methoxyaniline), 126.1 (C1 of phenyl at C6-pyrimidine), 124.8 (C-2, C-6 of phenyl at C6-pyrimidine), 121.0 (C4 of 4-methoxyaniline + C4 of 4-methoxyphenyl at C3-pyrazole), 120.9 (C-3, C-5 of phenyl at C6-pyrimidine), 116.5 (C1 of 4-methoxyphenyl at C3-pyrazole), 114.1 (C-2, C-6 of 4-methoxyaniline + C-2, C-6 of 4-methoxyphenyl at C3-pyrazole), 113.8 (C-3, C-5 of 4-methoxyaniline, C-3, C-5 of 4-methoxyphenyl at C3-pyrazole), 82.2 (C4 of phenyl at C6-pyrimidine), 55.7 (OCH₃), 30.4 (OCH₃); Anal. Calcd. for C₂₉H₂₁N₇O₃ (515.52): C, 67.56; H, 4.11; N, 19.02. Found: C, 67.74; H, 4.18; N, 19.26.

General method for the synthesis of 2-(5-Amino-4-cyano-1H-pyrazol-1-yl)-6-methyl/phenyl-4-(4-methoxyanilino)-pyrimidine-5-carbonitriles (10a,b)

A mixture of the hydrazine derivative **5a,b** (0.5 mmol) and 2-(ethoxymethylene) malonitrile (Ding et al. 1987) (0.061 g, 0.5 mmol) in absolute EtOH (5 ml) was heated under reflux for 5 h and left to cool to RT. The separated product was filtered, washed with petroleum ether, dried, and crystallized from EtOH.

2-(5-Amino-4-cyano-1H-pyrazol-1-yl)-6-methyl-4-(4-methoxyanilino)-pyrimidine-5-carbonitrile (10a) Yield 23%; mp 122–123 °C; IR ν/cm^{-1} : 3384 (NH), 3309 (NH₂), 2210 (C≡N), 1635 (C=N), 1564 (C=C-Ar), 1256, 1025 (ν_{as} and ν_{s} C–O–C); ¹H NMR (DMSO-d₆, 300 MHz) δ/ppm: 10.06 (s, 1H, NH-Ar), 7.86 (s, 2H, pyrazole-NH₂), 7.84 (s, 1H, C3-H pyrazole), 7.43 (d, 2H, Ar-Hs, *o*- to NH-Ar, $J = 8.76$ Hz), 6.97 (d, 2H, Ar-Hs, *o*- to OCH₃, $J = 8.88$ Hz), 3.80 (s, 3H, OCH₃), 2.56 (s, 3H, CH₃); ¹³C NMR (DMSO-d₆, 75 MHz) δ/ppm: 172.7 (2 × CN), 160.6 (C5), 157.0 (C4-pyrazole), 155.8 (C2), 154.1 (C6), 143.0 (C-4, C-1 of 4-methoxyaniline), 129.7 (C5-pyrazole), 125.9 (C4 of 4-methoxyaniline), 114.5 (C-2, C-6 of 4-methoxyaniline), 114.0 (C-3, C-5 of 4-methoxyaniline), 72.6 (C3-pyrazole), 55.2 (OCH₃), 23.3 (C6-CH₃); EIMS *m/z*: 347 (M⁺, 23), 346 (100), 331 (66), 197 (22), 196 (22), 92 (22); Anal. Calcd. for C₁₇H₁₄N₈O (346.13): C, 58.95; H, 4.07; N, 32.35. Found: C, 59.18; H, 4.12; N, 32.49.

2-(5-Amino-4-cyano-1H-pyrazol-1-yl)-6-phenyl-4-(4-methoxyanilino)-pyrimidine-5-carbonitrile (10b) Yield 21%; mp 200–201 °C; IR ν/cm^{-1} : 3384 (NH), 3300 (NH₂), 2223 (C≡N), 1644 (C=N), 1564 (C=C-Ar), 1247, 1040 (ν_{as} and ν_{s} C–O–C); ¹H NMR (DMSO-d₆, 400 MHz) δ/ppm: 10.06 (s, 1H, NH-Ar, D₂O-exchangeable), 7.91(d, 2H, Ar-Hs of

C6-phenyl, $J = 6.88$ Hz), 7.87 (s, 1H, C3-*H* pyrazole), 7.84, (s, 2H, pyrazole-NH₂, D₂O-exchangeable), 7.59–7.64 (m, 3H, Ar-*H*s of C6-phenyl), 7.46 (d, 2H, Ar-*H*s, *o*- to NH-Ar, $J = 8.60$ Hz), 7.00 (d, 2H, Ar-*H*s, *o*- to OCH₃, $J = 8.64$ Hz), 3.81 (s, 3H, OCH₃); Anal. Calcd. for C₂₂H₁₆N₈O (408.42): C, 64.70; H, 3.95; N, 27.44. Found: C, 64.94; H, 3.93; N, 27.61.

General method for the synthesis of 2-(4-amino-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-6-methyl/phenyl-4-(4-methoxyanilino)-pyrimidine-5-carbonitriles (11a,b)

A mixture of 2-(5-amino-4-cyano-1*H*-pyrazol-1-yl)-4-(4-methoxyanilino)-6-substituted-pyrimidine-5-carbonitrile **10a,b** (1 mmol) and formamide (2 ml) was heated under reflux for 2–3 h. The solution was cooled to RT and poured onto H₂O. The obtained precipitate was filtered, dried and crystallized from EtOH.

2-(4-Amino-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-6-methyl-4-(4-methoxyanilino)-pyrimidine-5-carbonitrile (11a) Yield 70%; mp 199–197 °C; IR ν/cm^{-1} : 3499 (NH₂), 3294 (NH), 2213 (C≡N), 1636 (C=N), 1549 (C=C-Ar), 1248, 1033 (ν_{as} and ν_{s} C–O–C); ¹H NMR (DMSO-*d*₆, 400 MHz) δ /ppm: 8.83 (s, 1H, NH-Ar, D₂O exchangeable), 7.45 (d, 2H, Ar-*H*s, *o*- to NH-Ar, $J = 9.0$ Hz), 7.00 (s, *br*, 2H, NH₂, D₂O-exchangeable), 6.87 (d, 2H, Ar-*H*s, *o*- to OCH₃, $J = 9.0$ Hz), 3.74 (s, 3H, OCH₃), 2.30 (s, 3H, CH₃); Anal. Calcd. for C₁₈H₁₅N₉O (373.37): C, 57.90; H, 4.05; N, 33.76. Found: C, 58.08; H, 4.12; N, 33.94.

2-(4-Amino-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-6-phenyl-4-(4-methoxyanilino)-pyrimidine-5-carbonitrile (11b) Yield 75%; mp 205–207 °C; IR ν/cm^{-1} : 3491(NH₂), 3296 (NH), 2211 (C≡N), 1649 (C=N), 1560 (C=C-Ar), 1242, 1034 (ν_{as} and ν_{s} C–O–C); ¹H NMR (DMSO-*d*₆, 400 MHz) δ /ppm: 8.94 (s, 1H, NH-Ar, D₂O exchangeable), 7.78 (d, *dist*, 2H, Ar-*H*s, *o*- to NH-Ar, $J = 9.16$ Hz), 7.48–7.56 (m, 5H, Ar-*H*s of C6-phenyl), 7.21 (s, *br*, 2H, NH₂, D₂O-exchangeable), 6.91 (d, 2H, Ar-*H*s, *o*- to OCH₃, $J = 8.92$ Hz), 3.78 (s, 3H, OCH₃); ¹³C NMR (DMSO-*d*₆, 400 MHz) δ /ppm: 170.5 (CN), 163.1 (C5), 162.6 (C4 of pyrazolopyrimidine), 156.5 (C2), 137.6 (C4), 132.0 (C1 of 4-methoxyaniline), 130.8 (C6, C-1 of C6-phenyl), 129.1 (C-3a, C-7a of pyrazolopyrimidine), 128.9 (C6 of pyrazolopyrimidine), 128.8 (C-2, C-6 of C6-phenyl), 128.7 (C-3, C-5 of C6-phenyl), 125.6 (C-4 of 4-methoxyaniline + C-4 of C6-phenyl), 118.1 (C-2, C-6 of 4-methoxyaniline), 114.0 (C-3, C-5 of 4-methoxyaniline), 78.0 (C3), 55.7 (OCH₃); EIMS m/z : M⁺ absent, 434 (M⁺-1, 2), 330 (26), 300 (29), 287 (26), 243 (23), 234 (28), 230 (25), 199 (28), 198 (23), 195 (33), 181 (26), 171 (40), 169 (26), 168 (29),

167 (39), 165 (28), 158 (82), 157 (37), 156 (30), 155 (31), 153 (89), 151 (42), 148 (41), 147 (35), 142 (28), 141 (45), 137 (40), 136 (100), 134 (43), 133(38), 129 (31), 127 (36), 120 (33), 119 (56), 115 (32), 114 (34), 108 (40), 107 (409), 105 (44), 104 (93), 103 (31), 102 (48); Anal. Calcd. for C₂₃H₁₇N₉O (435.44): C, 63.44; H, 3.94; N, 28.95. Found: C, 63.71; H, 3.92; N, 27.17.

General method for the synthesis of 4-(4-methoxyanilino)-6-methyl-2-[2-(substituted thiocarbamoyl)hydrazinyl]-pyrimidine-5-carbonitriles (12a,b)

The appropriate isothiocyanate derivative (1 mmol) was added to a well stirred suspension of the hydrazine derivative **5a** (0.27 g, 1 mmol) in absolute EtOH (20 ml). The reaction mixture was stirred at RT for 2 h during which time dissolution and reprecipitation occurred. The obtained precipitate was filtered, dried and crystallized from EtOH.

4-(4-Methoxyanilino)-6-methyl-2-[2-phenylthiocarbamoyl-hydrazinyl]-pyrimidine-5-carbonitrile (12a) Yield 86%; mp 130–132 °C; IR ν/cm^{-1} : 3400 (NH), 3268 (NH), 2226 (C≡N), 1700 (C=N), 1597 (C=C-Ar), 1551, 1334, 1199, 1039 (NH-C=S I, II, III, IV mixed vibrational bands), 1266, 1094 (ν_{as} and ν_{s} C–O–C); ¹H NMR (DMSO-*d*₆, 400 MHz) δ /ppm: 11.85 (s, 1H, NHNHC=S, D₂O-exchangeable), 10.28 (s, 1H, C2-NH, D₂O-exchangeable), 9.90 (s, 1H, NHC₆H₅, D₂O-exchangeable), 9.70 (s, *br*, 1H, C4-NH-Ar, D₂O-exchangeable), 7.14–7.55 (m, 5H, Ar-*H*s of phenylthiocarbamoyl), 7.54 (d, 2H, Ar-*H*s, *o*- to NH-Ar, $J = 7.76$ Hz), 7.15 (d, 2H, Ar-*H*s, *o*- to OCH₃, $J = 7.36$ Hz), 3.33 (s, 3H, O-CH₃), 2.32 (s, 3H, CH₃); Anal. Calcd. for C₂₀H₁₉N₇OS (405.14): C, 59.24; H, 4.72; N, 24.18. Found: C, 59.51; H, 4.79; N, 24.42.

4-(4-Methoxyanilino)-6-methyl-2-[2-(4-nitrophenyl)thiocarbamoylhydrazinyl]-pyrimidine-5-carbonitrile (12b) Yield 88%; mp 140–142 °C; IR ν/cm^{-1} : 3495 (NH), 3292 (NH), 2230 (C≡N), 1706 (C=N), 1640 (C=C-Ar), 1535, 1340 (ν_{as} and ν_{s} NO₂), 1560, 1356, 1204, 1094 (NH-C=S I, II, III, IV mixed vibrational bands), 1270, 1111 (ν_{as} and ν_{s} C–O–C); ¹H NMR (DMSO-*d*₆, 400 MHz) δ /ppm: 13.20 (s, 1H, NHC₆H₄-NO₂, D₂O-exchangeable), 11.62 (s, 1H, NHNHC=S NHC₆H₄, D₂O-exchangeable), 10.87 (s, 1H, C2-NH, D₂O-exchangeable), 9.60 (s, *br*, 1H, C4-NH-Ar, D₂O-exchangeable), 8.25 (d, 2H, Ar-*H*s *o*- to NO₂, $J = 7.08$ Hz), 8.23 (d, 2H, Ar-*H*s *m*- to NO₂, $J = 7.08$ Hz), 7.80 (d, 2H, Ar-*H*s, *o*- to NH-Ar, $J = 9.20$ Hz), 6.60 (d, 2H, Ar-*H*s, *o*- to OCH₃, $J = 9.20$ Hz), 3.43 (s, 3H, O-CH₃), 2.32 (s, 3H, CH₃); Anal. Calcd. for C₂₀H₁₈N₈O₃S (450.12): C, 53.32; H, 4.03; N, 24.87. Found: C, 53.59; H, 4.07; N, 25.03.

General method for the synthesis of 6-methyl-4-(4-methoxyanilino)-2-(2-(4-oxo-3-arylthiazolidin-2-ylidene)hydrazinyl)pyrimidine-5-carbonitriles (13a,b)

Ethyl bromoacetate (0.167 g, 1 mmol) and anhydrous NaOAc (0.12 g, 1.5 mmol) in EtOH (5 ml) were added to the solution of the appropriate thiosemicarbazide derivative **12a,b** and the mixture was heated under reflux for 3 h, concentrated to half its volume, allowed to attain RT and poured onto ice-cold H₂O (10 ml). The obtained precipitate was filtered, dried and crystallized from EtOH.

6-Methyl-4-(4-methoxyanilino)-2-(2-(4-oxo-3-phenylthiazolidin-2-ylidene)hydrazinyl)pyrimidine-5-carbonitrile (13a) Yield 22%; mp 199–201 °C; IR ν/cm^{-1} : 3359 (NH), 2211 (C≡N), 1742 (C=O), 1651 (C=N), 1585 (C=C–Ar), 1234, 1166 (ν_{as} and ν_{s} C–O–C); ¹H NMR (DMSO-*d*₆, 400 MHz) δ/ppm : 10.42 (s, *br*, 1H, C2-NH), 9.37 (s, 1H, NH-Ar), 7.08–7.50 (m, 5H, Ar-*H* of thiazolidinone-N-phenyl), 6.89 (d, 2H, Ar-*Hs*, *o*- to NH-Ar, *J* = 8.68 Hz), 6.68 (d, 2H, Ar-*Hs*, *o*- to OCH₃, *J* = 7.64 Hz), 4.18 (d, 1H, thiazolidinone-C5-*H_B*, *J* = 7.68 Hz), 3.77 (d, 1H, thiazolidinone-C5-*H_A*, *J* = 7.6 Hz), 3.72 (s, 3H, OCH₃), 2.43 (s, 3H, CH₃); ¹³C NMR (DMSO-*d*₆, 101 MHz) δ/ppm : 172.8 (CN), 169.5 (C=O), 161.1 (C5), 160.6 (C2), 156.6 (C1 of thiazolidinone-N-phenyl), 153.0 (thiazolidinone-C2), 148.1 (C6), 131.4 (C4), 129.8 (C1 of 4-methoxyaniline), 124.8 (C-2, C-6 of thiazolidinone-N-phenyl), 120.9 (C-2, C-6 of 4-methoxyaniline), 116.5 (C-4 of phenyl), 114.1 (C-3, C-5 of thiazolidinone-N-phenyl), 113.8 (C-3, C-5 of 4-methoxyaniline), 82.2 (C4 of thiazolidinone-N-phenyl), 55.7 (OCH₃), 30.4 (thiazolidinone-C5), 23.5 (CH₃); EIMS: 445 (M⁺, 31), 240 (100), 239 (53), 225 (23), 197 (16), 196 (22), 135 (13), 108 (22), 92 (17), 77 (49); Anal. Calcd. for C₂₂H₁₉N₇O₂S (445.50): C, 59.31; H, 4.30; N, 22.01. Found: C, 59.48; H, 4.38; N, 22.19.

6-Methyl-4-(4-methoxyanilino)-2-(2-(4-oxo-3-(4-nitrophenyl)thiazolidin-2-ylidene)hydrazinyl)pyrimidine-5-carbonitrile (13b) Yield 20%; mp 203–205 °C; IR ν/cm^{-1} : 3399 (NH), 2213 (C≡N), 1740 (C=O), 1651 (C=N), 1585 (C=C–Ar), 1489, 1339 (ν_{as} and ν_{s} NO₂), 124, 1176 (ν_{as} and ν_{s} C–O–C); ¹H NMR (DMSO-*d*₆, 400 MHz) δ/ppm : 10.54 (s, 1H, C2-NH), 9.44 (s, 1H, NH-Ar), 8.18 (d, 2H, Ar-*Hs*, *o*- to NO₂, *J* = 8.0 Hz), 7.41 (d, 2H, Ar-*Hs*, *o*- to NH-Ar, *J* = 8.0 Hz), 6.94 (d, 2H, Ar-*Hs*, *m*- to NO₂, *J* = 8 Hz), 6.85 (d, 2H, Ar-*Hs*, *o*- to OCH₃, *J* = 8.0 Hz), 4.29 (d, 1H, thiazolidinone-C5-*H_B*, *J* = 15.0 Hz), 3.85 (d, 1H, thiazolidinone-C5-*H_A*, *J* = 16.0 Hz), 3.73 (s, 3H, OCH₃), 2.43 (s, 3H, CH₃); ¹³C NMR (DMSO-*d*₆, 101 MHz) δ/ppm : 171.3 (CN), 168.8 (C=O), 160.0 (C5), 159.8 (C4 of thiazolidinone-N-nitrophenyl), 159.1 (C2), 156.6 (C1 of thiazolidinone-N-

nitrophenyl), 155.2 (thiazolidinone-C2), 142.5 (C6), 130.0 (C4), 125.2 (C-3, C-5 of thiazolidinone-N-nitrophenyl), 124.7 (C-2, C-6 of thiazolidinone-N-nitrophenyl), 124.8 (C1 of 4-methoxyaniline), 121.5 (C-2, C-6 of 4-methoxyaniline), 116.0 (C4 of 4-methoxyaniline), 113.5 (C-3, C-5 of 4-methoxyaniline), 82.2 (thiazolidinone-C5), 55.3 (OCH₃), 23.0 (CH₃); HRESIMS: M⁺+1 491.12363 (100) (calculated mass = 490.49); Anal. Calcd. for C₂₂H₁₈N₈O₄S (490.49): C, 53.87; H, 3.70; N, 22.85. Found: C, 54.03; H, 3.74; N, 23.03.

General method for the synthesis of 6-methyl-4-(4-methoxyanilino)-2-(3,4-diarylthiazol-2(3H)-ylidenehydrazinyl)pyrimidine-5-carbonitriles (14a–c, 15a–c)

The properly 4-substituted phenacyl bromide (1 mmol) was added to a solution of the corresponding thiosemicarbazide derivative **12a,b** and sodium acetate (0.164 g, 2 mmol) in EtOH (5 ml) and the reaction mixture was heated under reflux for 2 h, concentrated to half its volume then left to cool to RT. The obtained precipitate was filtered, washed with petroleum ether (40°–60°), dried and crystallized from EtOH.

6-Methyl-4-(4-methoxyanilino)-2-(3-phenyl-4-(4-bromophenyl)thiazol-2(3H)-ylidene hydrazinyl)pyrimidine-5-carbonitrile (14a) Yield 22%; mp 178–180 °C; IR ν/cm^{-1} : 3320 (NH), 2215 (C≡N), 1636 (C=N), 1570 (C=C–Ar), 1230, 1114 (ν_{as} and ν_{s} C–O–C); ¹H NMR (DMSO-*d*₆, 400 MHz) δ/ppm : 9.77, 9.60 (2s, 2 × 1H, C2-NH), 9.31, 8.84 (2s, 2 × 1H, NH-Ar), 6.85–8.18 (hump, 26 H, Ar-*Hs*), 6.79, 6.77 (2s, 2 × 1H, thiazoline-*H*), 3.76, 3.73 (2s, 2 × 3H, 2 × OCH₃), 2.34, 2.30 (2s, 2 × 3H, 2 × CH₃); HRESIMS: M⁺+1 = 584.08429 (9), M⁺+3 = 586.07745 (8); calculated mass = 583 (585); Anal. Calcd. for C₂₈H₂₂BrN₇O₂S (584.49): C, 57.54; H, 3.79; N, 16.77. Found: C, 57.70; H, 3.82; N, 16.89.

6-Methyl-4-(4-methoxyanilino)-2-(3-phenyl-4-(4-chlorophenyl)thiazol-2(3H)-ylidene hydrazinyl)pyrimidine-5-carbonitrile (14b) Yield 23%; mp 150–152 °C; IR ν/cm^{-1} : 3293 (NH), 2209 (C≡N), 1636 (C=N), 1573 (C=C–Ar), 1510, 1040 (ν_{as} and ν_{s} C–O–C); ¹H NMR (DMSO-*d*₆, 400 MHz) δ/ppm : 9.77, 9.52 (2s, 2 × 1H, C2-NH), 9.38, 8.84 (2s, 2 × 1H, NH-Ar), 6.74–7.67 (hump, 26 H, Ar-*Hs*), 6.50, 6.41 (2s, 2 × 1H, thiazoline-*H*), 3.76, 3.73 (2s, 2 × 3H, 2 × OCH₃), 2.34, 2.29 (2s, 2 × 3H, 2 × CH₃); ¹³C NMR (DMSO-*d*₆, 101 MHz) δ/ppm : 172.7, 172.0 (CN), 163.0, 162.2 (C5), 161.4, 160.9 (C2), 157.0, 156.7 (C6), 155.9, 155.7 (thiazoline-C2), 151.0, 150.9 (C4 of 4-chlorophenyl), 140.8, 140.6 (C1 of thiazoline-2-(or 3-)-phenyl), 133.4, 133.3 (C4), 133.2, 131.9 (C1 of 4-methoxyaniline), 130.0,

129.5 (C-3, C-5 of 4-chlorophenyl), 129.4, 129.3 (C-2, C-6 of thiazoline-2-(or 3-)-phenyl), 128.5, 127.9 (C1 of 4-chlorophenyl), 125.7, 125.6 (C-2, C-6 of 4-chlorophenyl), 123.8, 123.7 (C-2, C-6 of 4-methoxyaniline), 121.60, 121.5 (C-3, C-5 of thiazoline-2-(or 3-)-phenyl), 116.7, 116.4 (C4 of 4-methoxyaniline), 114.5, 114.1 (C4 of thiazoline-2-(or 3-)-phenyl), 113.9, 113.7 (C-3, C-5 of 4-methoxyaniline), 92.7, 91.5 (thiazoline-C4), 82.1, 81.3 (thiazoline-C5), 55.8, 55.7 (OCH₃), 23.4 (CH₃); HRESIMS: M⁺ + 1 = 540.1364 (100), M⁺ + 3 = 542.1340 (39) (calculated mass = 540.04); Anal. Calcd. for C₂₈H₂₂ClN₇OS (540.04): C, 62.27; H, 4.11; N, 18.16. Found: C, 62.41; H, 4.17; N, 18.34.

6-Methyl-4-(4-methoxyanilino)-2-(3-phenyl-4-(4-nitrophenyl)-2(3H)-ylidene-hydrazinyl)pyrimidine-5-carbonitrile (14c) Yield 25%; mp 166–167 °C; IR ν/cm⁻¹: 3411 (NH), 2213 (C≡N), 1636 (C=N), 1593 (C=C-Ar), 1415, 1347 (ν_{as} and ν_s NO₂), 1237, 1114 (ν_{as} and ν_s C-O-C); ¹H NMR (DMSO-d₆, 400 MHz) δ/ppm: 9.83, 9.43, (2s, 2 × 1H, C2-NH), 9.29, 8.95 (2s, 2 × 1H, NH-Ar), 6.53–8.38 (hump, 28 H, thiazoline-H + Ar-Hs), 3.76, 3.74 (2s, 2 × 3H, 2 × OCH₃), 2.35, 2.29 (2s, 2 × 3H, 2 × CH₃); HRESIMS: M⁺ + 1 = 551.15948 (12) (calculated mass = 550.59); Anal. Calcd. for C₂₈H₂₂N₈O₃S (550.59): C, 61.08; H, 4.03; N, 20.35. Found: C, 61.35; H, 4.11; N, 20.57.

6-Methyl-4-(4-methoxyanilino)-2-(3-(4-nitrophenyl)-4-(4-bromophenyl)thiazol-2(3H)-ylidenehydrazinyl)pyrimidine-5-carbonitrile (15a) Yield 23%; mp 183–182 °C; IR ν/cm⁻¹: 3305 (NH), 2210 (C≡N), 1620 (C=N), 1580 (C=C-Ar), 1515, 1342 (ν_{as} and ν_s NO₂), 1235, 1040 (ν_{as} and ν_s C-O-C); ¹H NMR (DMSO-d₆, 400 MHz) δ/ppm: 10.67, 10.16 (2s, 2 × 1H, C2-NH), 9.41, 9.13 (2s, 2 × 1H, NH-Ar), 6.88–8.19 (hump, 24H, Ar-Hs), 6.70, 6.45 (2s, 2 × 1H, thiazoline-H), 3.76, 3.74 (2s, 2 × 3H, 2 × OCH₃), 2.34, 2.29 (2s, 2 × 3H, 2 × CH₃); ¹³C NMR (DMSO-d₆, 101 MHz) δ/ppm: 172.5, 172.1 (CN), 162.6, 161.9 (C5), 161.5, 161.1 (C2), 157.5, 157.3 (C6), 157.0, 156.9 (thiazoline-C2), 143.5, 143.3 (C4 of 4-bromophenyl), 140.9, 140.5 (C4 of thiazoline-2-(or 3-)-4-nitrophenyl), 131.7, 131.5 (C4), 130.9, 130.7 (C1 of 4-methoxyaniline), 129.8, 129.6 (C-3, C-5 of thiazoline-2-(or 3-)-4-nitrophenyl), 126.1, 125.8 (C-3, C-5 of 4-bromophenyl), 125.6, 125.5 (C1 of 4-bromophenyl), 122.5, 122.4 (C-2, C-6 of thiazoline-2-(or 3-)-4-nitrophenyl), 122.3, 122.2 (C-2, C-6 of 4-bromophenyl), 122.1, 121.8 (C-2, C-6 of 4-methoxyaniline), 116.7, 114.0 (C4 of 4-methoxyaniline), 114.5, 114.1 (C1 of thiazoline-2-(or 3-)-4-nitrophenyl), 114.0, 113.8 (C-3, C-5 of 4-methoxyaniline), 93.3, 92.0 (thiazoline-C4), 82.0, 81.4 (thiazoline-C5), 55.8, 55.7 (OCH₃), 23.4 (CH₃); Anal. Calcd. for C₂₈H₂₁BrN₈O₃S (629.49): C, 53.42; H, 3.36; N, 17.80. Found: C, 53.69; H, 3.34; N, 18.02.

6-Methyl-4-(4-methoxyanilino)-2-(3-(4-nitrophenyl)-4-(4-chlorophenyl)thiazol-2(3H)-ylidenehydrazinyl)pyrimidine-5-carbonitrile (15b) Yield 25%; mp 185–186 °C; IR ν/cm⁻¹: 3293 (NH), 2209 (C≡N), 1610 (C=N), 1573 (C=C-Ar), 1415, 1338 (ν_{as} and ν_s NO₂), 1230, 1034 (ν_{as} and ν_s C-O-C); ¹H NMR (DMSO-d₆, 400 MHz) δ/ppm: 9.86, 9.45 (2s, 2 × 1H, C2-NH), 9.41, 9.13 (2s, 2 × 1H, NH-Ar), 6.96–8.21 (hump, 24H, Ar-Hs), 6.50 (s, 1H, thiazoline-H), 3.73, 3.70 (2s, 2 × 3H, 2 × OCH₃), 2.60, 2.30 (2s, 2 × 3H, 2 × CH₃); EIMS m/z: 586 (M⁺, 17), 584 (43), 449 (26), 448 (21), 447 (71), 417 (19), 416 (73), 401 (41), 391 (13), 389 (19), 331 (30), 286 (14), 285 (13), 284 (33), 255 (57), 254 (64), 240 (100), 239 (93), 225 (25), 224 (20), 218 (14), 214 (20), 211 (24), 197 (32), 170 (40), 168 (90), 139 (33), 134 (27), 133 (41), 108 (27), 90 (32); Anal. Calcd. for C₂₈H₂₁ClN₈O₃S (584.11): C, 57.48; H 3.62; N, 19.15. Found: C, 57.62; H, 3.60; N, 19.38.

6-Methyl-4-(4-methoxyanilino)-2-(3,4-(4-nitrophenyl)thiazol-2(3H)-ylidenehydrazinyl)pyrimidine-5-carbonitrile (15c) Yield 26%; mp 190–191 °C; IR ν/cm⁻¹: 3411 (NH), 2213 (C≡N), 1636 (C=N), 1593 (C=C-Ar), 1509, 1347 (ν_{as} and ν_s NO₂), 1237–1230, 1114–1034 (ν_{as} and ν_s C-O-C); ¹H NMR (DMSO-d₆, 400 MHz) δ/ppm: 9.88, 9.73 (2s, 2 × 1H, C2-NH), 9.44, 9.21 (2s, 2 × 1H, NH-Ar), 6.97–8.24 (hump, 24H, Ar-Hs), 6.66 (s, 1H, thiazoline-H), 3.74, 3.72 (2s, 2 × 3H, 2 × OCH₃), 2.33, 2.28 (2s, 2 × 3H, 2 × CH₃); Anal. Calcd. for C₂₈H₂₁N₉O₅S (595.59): C, 56.47; H, 3.55; N, 21.17. Found: C, 56.59; H, 3.59; N, 21.39.

Pharmacological studies

Drugs, chemicals, and materials

Nifedipine was obtained from Amriya Pharmaceutical Industries, Alexandria-Cairo Desert Road Km 25, Amriya, Alexandria, Egypt. Other chemicals such as pentobarbital sodium, heparin, dimethylsulfoxide (DMSO), freshly mixed heparinized saline, physiological solutions were of analytical grade. i.v. Catheters were of 20GA, 22 GA, 24 GA calibers.

Animals

White New Zealand rabbits (2–2.5 kg) of either sex were obtained from Animal House, Faculty of Medicine, Assiut University. Rabbits were kept in cages in Faculty of Medicine, Al-Azhar University, Assiut Branch, Assiut, housed at ordinary RT, exposed to natural daily light-dark cycles, fed with standard laboratory diet and allowed for tap H₂O ad libitum.

In vivo experiments

Forty-two normotensive white New Zealand rabbits (2–2.5 kg) of either sex were used for this assay and six rabbits were used for each group.

In vivo assessment: hypotensive activity on normotensive anesthetized rabbits

Normotensive rabbits were anesthetized with pentobarbital sodium (30 mg/kg, i.v.). They were placed on their backs on the operating table while legs were fixed and heads pinned down. Trachea were exposed and cut and plastic cannula were inserted and firmly tied in place. The common carotid artery lying in the neck between the lateral bundle of muscle (*longus capitis*) and the trachea was exposed by separating the two muscle bundles of the sternomastoid and the sternothyroid. The carotid artery was separated from the nerves and veins and carefully freed of connective tissue. A venous cannula filled with heparin to prevent thrombus formations was carefully inserted and a thread was tied on it. The cannula was connected to a pressure transducer via universal oscillograph (Harvard apparatus, ser. No. K10542) for blood pressure records. On the opposite side, a venous cannula was inserted into the external jugular vein and tied with thread for injection of 29 tested compounds followed by heparinized saline (0.90% w/v NaCl). The mean arterial blood pressure was calculated using the formula: $MAP = DBP + 1/3 (SBP - DBP)$, where MDP = mean arterial blood pressure, DBP = diastolic blood pressure, and SBP = systolic blood pressure.

In vitro experiments

Seven white New Zealand rabbits (2–2.5 kg) of either sex were starved and allowed free access to H₂O for 24 h prior to experiments then slaughtered at the day of experiment to isolate the heart and aorta.

Calcium channel blockade activity

Calcium channel blockade activity procedure was carried out in accordance to the previously reported technique (Kaur and Kaur 2013; Girgis et al. 2006, 2008). Compounds **5a**, **5b**, **9b**, and **9c** which elicited high hypotensive activity were used for assessment of the calcium channel blockade activity. In order to check for antagonistic effects, contractions were induced with potassium chloride (67 mmol/L). After thorough washing out, this process was repeated until the amplitude of the contraction became constant. The substances to be tested were investigated using the single-dose technique. KCl contractions were induced after addition of the substances at 10^{-4} M

concentration and 10 min exposure time. Between administrations of the individual substances, the preparation was washed until the initial situation had been re-established and the potassium chloride contractions were induced. The contractions were enrolled by FTO3 force–displacement transducer, and recorded on a polygraph (Kaur and Kaur 2013; Girgis et al. 2006, 2008). When response reached its plateau, response of aorta rings to solutions of **5a**, **5b**, **9b**, and **9c** was done and each ring was serially washed after obtaining the maximum response to reach the baseline and equilibrated. At this point, another compound solution was added.

Effect of test compounds on the rabbits heart rates

Hearts of rabbits were prepared according to the previously reported method (Spáčilová 2007; Burn 1952). Aorta was dissected off, freed from all other connecting vessels, carefully tied and cut. Threads were attached to the ventricle by means of a small clip and connected to a special transducer via universal oscillograph (Harvard apparatus, ser. No. K10542) for heart activity records. Test compounds **5a**, **5b**, **9b**, and **9c** were added to the cannulae containing the perfusion fluid and entered aorta to reach the coronaries. Aorta was tied into the glass cannula of the perfusion apparatus. Oxygenated Locke solution at 37 °C was allowed in from a reservoir maintained at a constant pressure. That keeps the aortic valve closed while the fluid can pass through the coronary vessels and exit from the inferior vena cava to be collected in a container (Spáčilová 2007; Burn 1952).

In vitro preliminary screening of eNOS activation in aorta and histopathological changes

Materials and method

Chemicals

Endothelial nitric oxide synthase antibody (eNOS) was purchased from Thermo Scientific Co.

Animals

Eight adult male rats weighing about 220–250 g were used in the present work. They were purchased from Assiut University Animal Breeding Unit. Animals were kept in a controlled light room with photoperiod of 12 h dark and 12 h light (dark–light cycle 12:12) with lights ON from 6:00 to 18:00 h at a temperature of 28 ± 2 °C. All animals were given free access to standard laboratory chow and tap water.

Rats were randomly divided into 4 groups, 2 rats each. Group (I) served as control group and used for hematoxylin and eosin staining and eNOS immunostaining while Group (II) and (III) received compounds **5a** and **5b** respectively by intra-peritoneal injection in a dose of 30 mg/kg body weight for eNOS immunostaining. Finally, Group (IV) served as positive control and received L-NAME an inhibitor for eNOS synthesis in a dose of 30 mg/kg body weight by intra-peritoneal injection for eNOS immunostaining.

Tissue preparation

Animals were anesthetized with ether, fixed by intracardiac perfusion with 10% formaldehyde. Aorta specimens were rapidly dissected immediately, and immersed into 10% formaldehyde solution to continue fixation 2 more days (pH 7.2). Specimens were processed to prepare paraffin blocks through dehydration in ascending series of EtOH, cleared in methyl benzoate and embedded in paraffin wax. Paraffin sections (5 μ m) were cut using a microtome mounted on glass slides, for histological examination with hematoxylin–eosin stain and immunohistochemical staining.

Histological and histopathological examinations

Immunohistochemistry

For eNOS immunohistochemistry: After fixation in 10% neutral formalin for 2 days, dehydration, clearing, and embedding in paraffin soon followed. Paraffin sections were cut at 5 μ m and stained with avidin–biotin peroxidase technique. Sections underwent deparaffinization and rehydration by descending grades of EtOH (100, 90, and 70%). They were treated with 0.01 M citrate buffer (pH 6.0) for 10 min to unmask antigen and incubated in 0.3% H₂O₂ for 30 min to abolish endogenous peroxidase activity before blocking with 5% horse serum for 1–2 h. Slides were incubated with the primary antibody (1:100 monoclonal mouse anti-eNOS) at 4 °C for 18–20 h, then washed and incubated with biotinylated secondary antibodies and then with avidin–biotin complex. Finally, sections were developed with diaminobenzidine (DAB) chromogen. Slides were counter stained with Mayer's hematoxylin, dehydrated, cleared, and mounted. eNOS positive cells appeared with brown cytoplasm while nuclei appeared blue.

Results and discussion

Chemistry

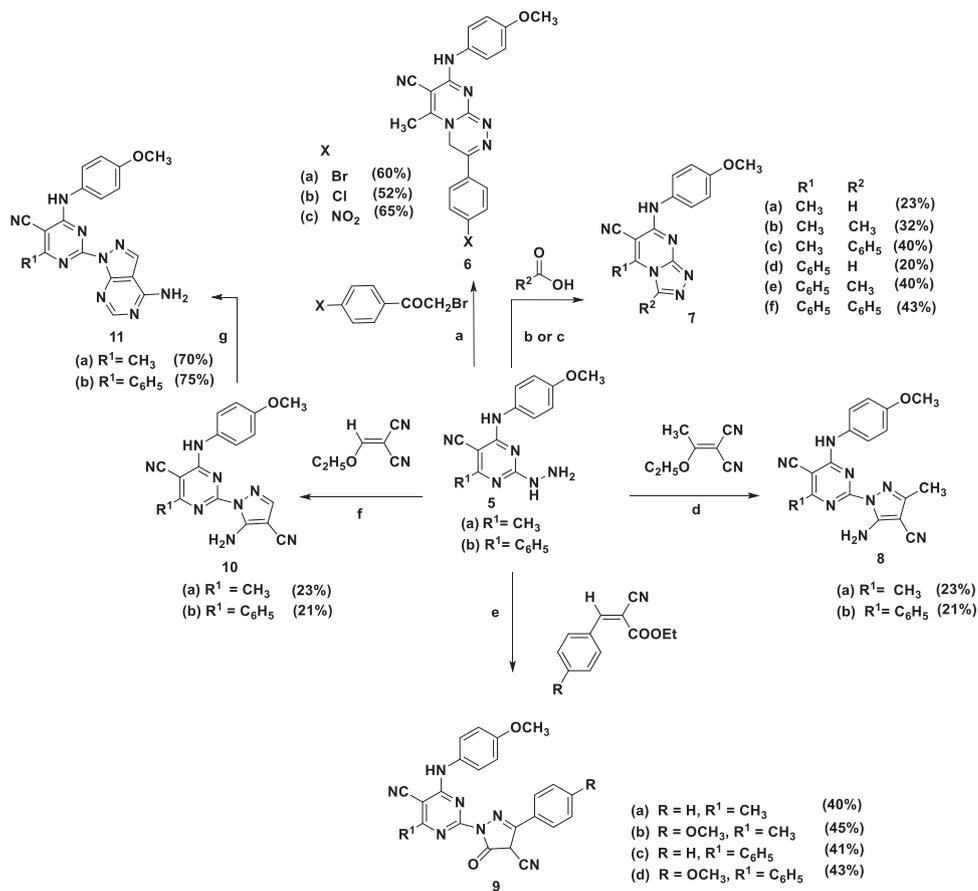
The designed pyrimidine derivatives were obtained through the synthesis of hydrazinyl pyrimidine derivatives **5a,b** as

described (Scheme 1). 2-Methylthio derivatives **2a,b** were prepared by heating a mixture of compounds **1a** or **1b** (Jones 1952), *S*-methylthiourea sulfate and anhydrous AcONa in DMF. Heating compounds **2a,b** with 2.5 molar equivalents of POCl₃ at RT in presence of *N,N*-dimethylaniline furnished compounds **3a,b**. This modified method was better than the reported one (Ding et al. 1987) avoiding the use of excess of POCl₃. Compounds **4a,b** were prepared by heating under reflux the corresponding 4-chloro derivatives **3a,b** with 4-methoxyaniline in EtOH in presence of K₂CO₃ (Atwal et al. 1987). Hydrazine hydrate was allowed to react with the *S*-methyl derivatives **4a,b**, where upon the good leaving sulfanyl group was successfully displaced (Spáčilová et al. 2007) furnishing the 2-hydrazinyl derivatives **5a,b** (Scheme 1).

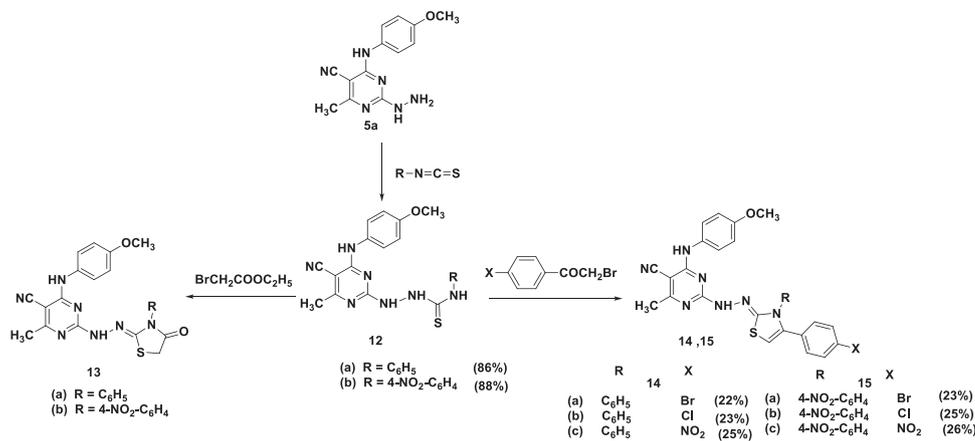
It was of interest to extend the scope of such valuable transformation allowing the introduction of considerable functionalities at C2 of Biginelli derived pyrimidine core. In this context, hydrazine derivatives **5a,b** reacted with different reagents to install a variety of heterocycles linked with pyrimidine system as illustrated in Schemes 2 and 3. Addition of phenacyl bromides to the key intermediate **5a** in EtOH furnished the desired compounds **6a–c**, Scheme 2. Triazolopyrimidine derivatives **7a–f** were successfully prepared by heating the hydrazines **5a,b** with aliphatic carboxylic acids where in situ cyclization occurred. In case of the less reactive benzoic acid, POCl₃ was necessary to catalyze the reaction, Scheme 2. Compounds **8a,b** and **10a,b** were obtained in a fairly good yield upon treatment of the hydrazines **5a,b** with appropriate acceptors as 2-(1-ethoxyethylene)malononitrile (Jones 1952) or 2-(ethoxymethylene)malononitrile (Ding et al. 1987), in boiling EtOH, Scheme 2. Heating under reflux compounds **10a,b** with excess formamide gave the corresponding 2-4-amino-1*H*-pyrazolo[3,4-*d*]pyrimidine derivatives **11a,b**, Scheme 2. A mixture of the hydrazine derivatives **5a,b** and ethyl 2-cyano-3-arylacrylates (Hussain et al. 1985) were reacted in boiling EtOH, however, unlike previously evidenced, the second attack of *N*¹ of hydrazine took place on the carbonyl ester rather on the nitrile C giving rise to the postulated pyrazolonitrile product **9a–d** instead of the pyrazole ester initially reported in literature (Aggarwal et al. 2011; Bakr and Kamal 2012). Confirmatory evidence for structures **9a–d** was proved by spectral data (IR, ¹H NMR and ¹³C NMR and MS).

Under neutral conditions, the hydrazine derivatives **5a,b** were allowed to react with phenyl or *p*-nitrophenyl isothiocyanate affording products **12a,b** in good yields (Scheme 3). Condensation of **12a,b** with ethyl bromoacetate in boiling EtOH containing anhydrous NaOAc afforded the corresponding thiazolidinone derivatives **13a,b**, Scheme 3. It was postulated that reaction proceeded via *S*-alkylation followed by elimination of H₂O and cyclization. Reacting

Scheme 2 Synthetic pathways to pyrimidotriazines **6a–c**, triazolopyrimidines **7a–f**, pyrazolopyrimidines **8a,b**, **9a–d**, and **10a,b** and pyrazolopyrimidinylpyrimidines **11a,b**



Scheme 3 Synthetic pathways to thiosemicarbazide derivatives **12a,b**, thiazolidinone derivatives **13a,b**, thiazoline derivatives **14a–c** and **15a–c**



12a,b with various phenacyl bromides provided the thiazoline derivatives **14a–c** and **15a–c**. However, 2 positional isomers might be possible (Bonde and Gaikwad 2004). ¹H NMR analysis confirmed the presence of a mixture of the 2 isomers as indicated by duplication of many of ¹H and ¹³C NMR signals.

Pharmacological screening: calcium channel blockade activity

Several in vitro and in vivo techniques are available for evaluating calcium channel blocking activity (Vogel 2008).

Table 1 Effect of nifedipine and selected test compounds (mg/kg, i.v.) in normotensive anesthetized rabbits represented by change in MAP (mmHg)

Cpd No.	ABP (mmHg) as mean \pm SE	Cpd No.	ABP (mmHg) as mean \pm SE
Normal	92.2 \pm 0.86	9c	51.8 \pm 0.37* [#]
Nifedipine	84.2 \pm 0.58*	9d	56 \pm 0.70* [#]
5a	51.4 \pm 0.74* [#]	10c	91.6 \pm 0.81 [#]
5b	51.4 \pm 0.6* [#]	11b	71.8 \pm 0.37* [#]
7b	91.6 \pm 0.81 [#]	14c	91.8 \pm 0.66 [#]
7f	91.4 \pm 0.8 [#]	15b	78.2 \pm 0.66* [#]
8b	66.6 \pm 0.55* [#]	15c	92.0 \pm 0.54 [#]
9b	55.2 \pm 0.86* [#]		

Each value represents the mean \pm SE (standard error) of 6 experiments Nifedipine (0.01 M) caused 8 mmHg drop in arterial blood pressure

Results were significant at $P < 0.01$ according to Tukey–Kramer test

*Significant difference from the normal rabbits ($P < 0.01$)

[#]Significant difference from rabbits treated with nifedipine ($P < 0.01$)

In vitro studies are considered rapid useful tools for primary screening and various smooth muscle preparations such as rabbit jejunum (Taqvi et al. 2006), rat ileum (Taqvi et al. 2006), rat colon (Hadizadeh et al. 2008), and guinea pig trachea (Yu et al. 1994) were utilized as testing models. Moreover, whole animal experiments provide reliable assessment of their various in vivo effects. For evaluation of their cardiovascular activity, the main therapeutic value of CCBs, different models were employed, of which monitoring their effect on arterial blood pressure in hypertensive or normotensive dogs (Guarneri et al. 1997) or rabbits (Jeanneau et al. 1992).

In the current pharmacological assessment, three phases were established for in vitro and in vivo evaluation of the newly synthesized compounds: (1) Thirteen selected compounds were investigated for preliminary evaluation of hypotensive activity in normotensive anesthetized rabbits. (2) Active compounds from the previous screening were tested for calcium channel blockade on preparations of rabbit aortae. (3) Further, the same active compounds were evaluated for their effect on the rabbits heart rate.

Effect of tested compounds on mean arterial blood pressure (MABP)

Preliminary results of biological evaluation for synthesized compounds on mean arterial blood pressure (MABP) were examined and presented in Table 1 and Fig. 4. Among the tested compounds, results showed that MABP in rabbits treated with nifedipine decreased (84.2 \pm 0.58 mmHg) significantly ($P < 0.01$) in comparison with normal rabbits (92.2 \pm 0.86 mmHg). Compounds **5a**, **5b**, **8b**, **9b–d**, **11b**, and **15b** showed a decrease in MABP in the range

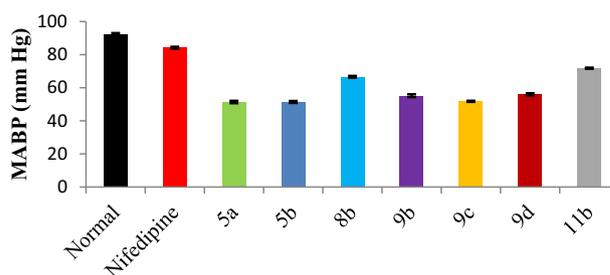


Fig. 4 Effect of nifedipine and compounds **5a**, **5b**, **8b** and **9b–d**, **11b** on MABP

51.4–78.2 mmHg in rabbits in comparison with nifedipine-treated rabbits. Among the compounds with hypotensive effects, we selected compounds **5a**, **5b**, **9b**, and **9c** for further assessment as CCBs.

Calcium channel blockade activity

As previously reported by other investigators (Zorkun et al. 2006), smooth muscles depend on calcium influx for contraction and although their underlying mechanism is somewhat different, inhibition of calcium channel influx into smooth muscles by CCBs leads to relaxation. Therefore, compounds **5a**, **5b**, **9b**, and **9c** which elicited high hypotensive activity were further evaluated for their calcium channel blockade activity as indicated by their effects on KCl-stimulated rabbit aortae. Nifedipine was included in all tests as the reference drug (Table 2 and Fig. 5). Results obtained from nifedipine, **5a**, **5b**, **9b**, and **9c**, indicated that the relaxant response of the aorta of the rabbits treated with test compounds increased significantly ($P < 0.01$) in comparison to nifedipine-treated rabbits aortae, as shown in Table 2 and Fig. 5. As shown, the maximum relaxation values of all tested compounds were much more superior when compared with that of nifedipine. In particular, the hydrazine derivatives **5a** and **5b** exhibited the highest activities showing 89.2% relaxation while all other tested compounds **9b** and **9c** showed 77–74.4% relation in comparison with nifedipine (57.6% relaxation).

Effect of nifedipine and compounds **5a**, **5b**, **9b**, and **9c** on heart rates

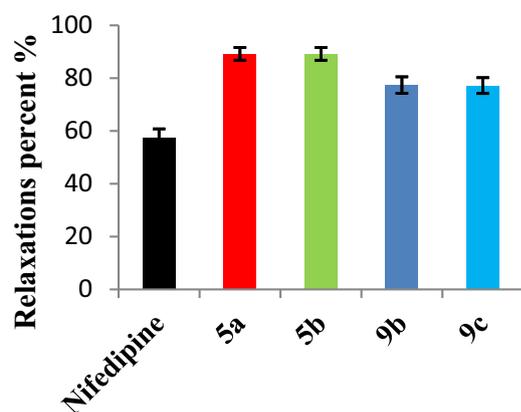
Active candidates that elicited significant lowering of blood pressure in normotensive rabbits in particular compounds **5a**, **5b**, **9b**, and **9c** were further evaluated for their effects on heart rates. Results of this study showed that, there is no significant difference ($P > 0.05$) between heart rates of nifedipine-treated rabbits and normal rabbits, but there were a significant difference ($P < 0.01$) on the heart rates between normal and nifedipine treated rabbits from one side and rabbits treated with test compounds from the other side. The

Table 2 Effect of nifedipine and compounds **5a**, **5b**, **9b**, and **9c** on the relaxant response of rabbit aortae

Groups	Nifedipine	5a	5b	9b	9c
Relaxation%	57.6 ± 3.20	89.2 ± 2.4 [#]	89.2 ± 2.41 [#]	77.4 ± 3.12 [#]	77.2 ± 3 [#]

Each value represents the mean ± SE (standard error) of 6 experiments

[#]Significant difference from rabbits treated with nifedipine ($P < 0.01$)

**Fig. 5** Effect of nifedipine and compounds **5a**, **5b**, **9b**, and **9c** on the relaxant response of rabbit aortae

effect of the test compounds on rabbit heart rate indicated that all compounds possessed a significant mean percentage decrease on rabbit's heart rate as shown in Table 3 and Fig. 6.

Correlation between *in vivo* hypotensive activity in normotensive anesthetized rabbits (Table 1) and *in vitro* calcium channel blockade activity as indicated by relaxant response of rabbit aortae (Table 2) indicated that compounds **5a**, **5b**, **9b**, **9c** exhibited promising lowering on MABP, calcium channel blocking activity accompanied by decrease in heart rate while compounds **8b**, **8d** elicited good lowering on MABP. In addition, compounds **7b**, **8a**, **11b**, **15b** showed moderate lowering on MABP. Such compounds could be further investigated to constitute a special class of compounds for treatment of hypotension. Meanwhile, **6c**, **7b**, **7f**, **14c**, **15b**, **15c** could be considered as inactive compounds.

Histopathological studies

The endothelium of blood vessels including aorta plays a modulator role in the basal and dynamic regulation of blood vessel diameter by releasing endothelium-derived nitric oxide (NO) (Zhao et al. 2015). In the blood vessel wall, eNOS has a protective function in the cardiovascular system attributable to NO production. Once NO is produced in endothelial cells, it diffuses across the vascular smooth muscle cell membranes and, through a special mechanism, promotes vascular relaxation (Zhao et al. 2013). In addition,

endothelium-derived NO may be involved in preventing the progression of age-related vascular diseases (Moncada and Higgs 2006, Pacher et al. 2007, Vanhoutte 2009; Vanhoutte et al. 2009).

Immuno-staining of the aortic sections of the four groups showed the followings changes.

Light microscopic examination of aortic section of the control rat group stained with hematoxylin and eosin showed the three layers of the aortic wall (Fig. 7). Control group showed mild immune-positive staining in the aortic endothelial cells of the intima and very few in adventitia (Fig. 8). In this study, compounds **5a** and **5b** were evaluated upon modulation blood pressure in an animal model of hypertension through its effect upon eNOS expression in the endothelium of aorta. Compounds **5a** and **5b** showed dense staining of the aortic endothelium in comparison to control group (Figs 9 and 10) respectively. The adventitia of **5a** and **5b** treated groups had immune-positive staining in the endothelial lining of blood vessels but seems to be more intense in the phenyl **5b** treated group (Figs 9 and 10). Compound **5b** induced increase in eNOS expression in the aorta as confirmed histologically in this study (Fig. 10). Intense eNOS expression localized to the aortic endothelium was observed with compound **5b** more than compound **5a** (Fig. 9). L-NAME (N ω -Nitro-L-arginine methyl ester hydrochloride) treated group as inhibitor of eNOS (positive control) showed no immunostaining of eNOS in the endothelial cells or other parts of the aorta (Fig. 11).

Structure–activity relationship (SAR) studies

As can be seen in the most active compounds, **5a**, **5b**, the most favorable structural elements contributing favorably to binding –NHNH₂ is capable of acting as H-bond acceptor/donor bound in a complementary region of the receptor containing amino acid residues with opposite features. Compound bearing a C6-methyl group **5a** exhibited a slight increase in inhibition activity. These results implied that electron donating groups at this position seemed to be beneficial to inhibitory activity (more than phenyl group). Thus, it was reasonable to presume that size of substituent at C6 is important to the activity of the target compounds. Besides, the promising activity of some compounds containing attached heterocyclic pyrazoline ring as **9b**, **9c** indicates that, while steric effects are important characteristics for activity, electronic and lipophilic effects are

Table 3 Effect of nifedipine and compounds **5a**, **5b**, **9b**, and **9c** on heart rates

Groups	Normal	Nifedipine	5a	5b	9b	9c
Heart rate/min	76 ± 1.30	74.6 ± 1.07	54 ± 0.70* [#]	52 ± 0.70* [#]	61.2 ± 0.58* [#]	60 ± 0.70* [#]

Each value represents the mean ± SE (standard error) of 6 experiments

*Significant difference from the normal rabbits ($P < 0.01$)

[#]Significant difference from rabbits treated with nifedipine ($P < 0.01$)

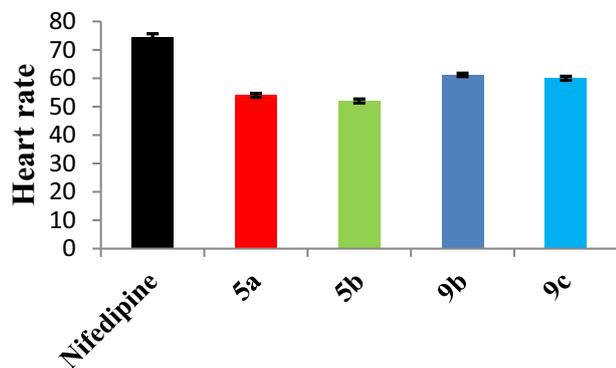


Fig. 6 Effect of nifedipine and compounds **5a**, **5b**, **9b**, and **9c** on the heart rates

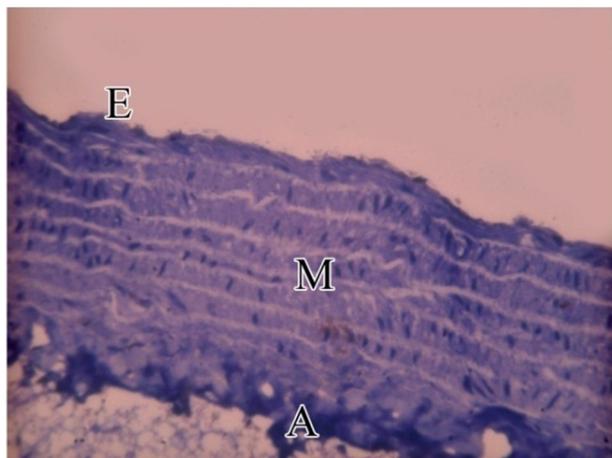


Fig. 7 Photomicrograph of a control adult rat aorta showing intimal endothelium (E) layer, the media (M), and the adventitia (A). H&E (400)

consistent also to contribute well for activity as the heterocyclic moiety might play a favorable role in overall binding and therefore in recognition area on the receptor. Such compounds, bearing a non-chiral planar pyrimidine ring would adopt the same total volume and polar surface area present in similar DHPs and DHPMs known to exhibit antihypertensive activity and indicated that introducing polar substituents at this position (NH) near the vicinity of C4 may lead to even better activity. Since all compounds have a common scaffold in their structures, observed SAR is mainly due to the variation of other functionalities on this

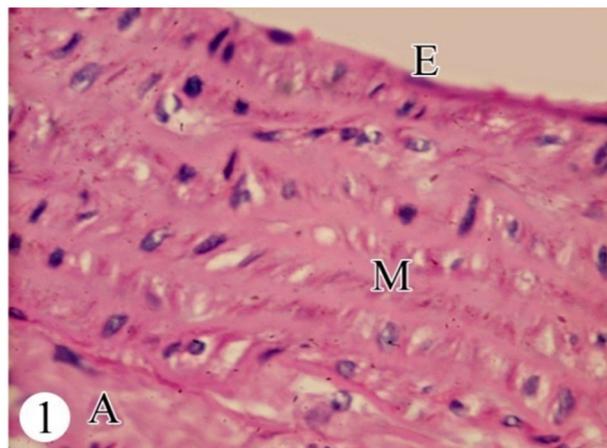


Fig. 8 Photomicrograph of a control adult rat aorta immunostained with anti-eNOS, a marker for e-NOS enzyme, showing immunopositive staining of the endothelium (eNOS immunostaining ×400). Inset: few immune-positive stained endothelial cells of adventitial blood vessels (↑) (eNOS immunostaining ×1000)

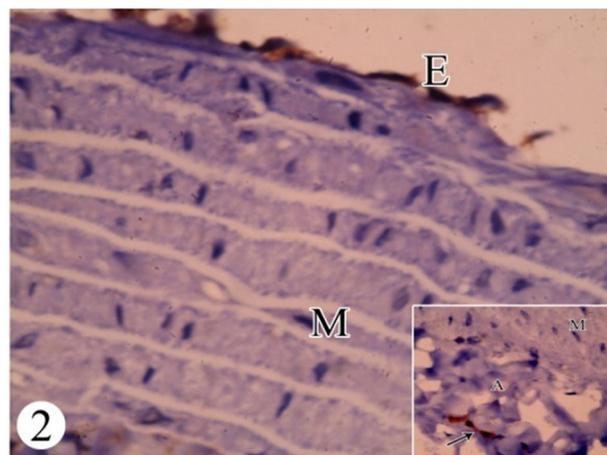


Fig. 9 Photomicrograph of adult rat aorta treated with **5a** and immunostained with anti-eNOS showing immunopositive staining of endothelium (E) more or less similar to control rat section (eNOS immune-staining ×400). Inset: more immune-positive stained endothelial cells of adventitial blood vessels (brown color) (eNOS immunostaining ×1000)

scaffold. Variation of the tail on the main scaffold at C2 is responsible for variation in activity potential although all structural features are taking part in the activity. Derivatives including versatile functionalities at position 2 varied

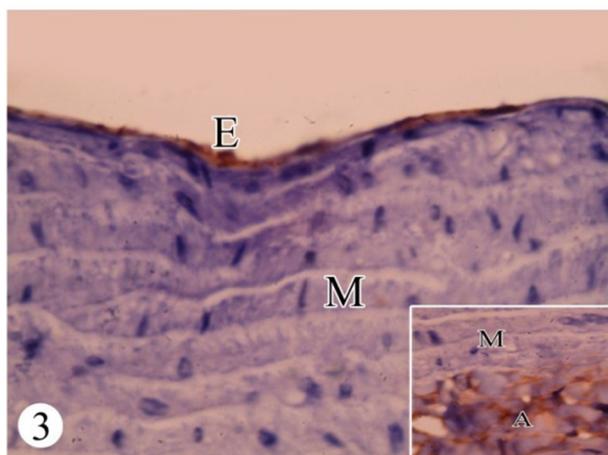


Fig. 10 Photomicrograph of adult rat aorta-treated with **5b** and immunostained with anti-eNOS showing dense immune-positive staining of endothelium (E) than control rat section (eNOS immunostaining $\times 400$). Inset: dense immune-positive stained endothelial cells of adventitial blood vessels (brown color) (eNOS immunostaining $\times 1000$)

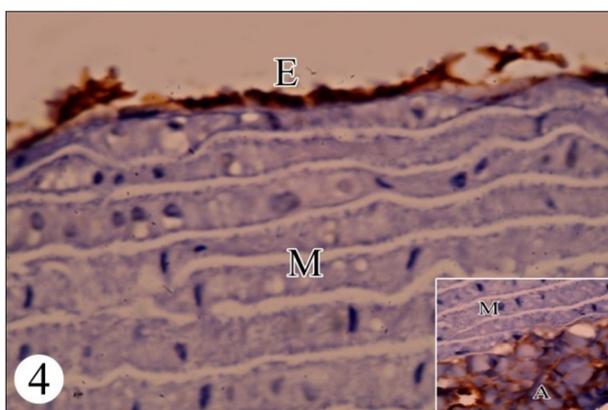


Fig. 11 Photomicrograph of adult rat aorta; a positive control (treated with L-NAME drug) immunostained with anti-eNOS, a marker for eNOS, showing no immuno-staining (eNOS immunostaining $\times 400$)

between simple flexible hydrazine derivatives, planar or non-planar aromatic heterocycles, and fused ring systems. Variation of substituents on C2-pyrimidine ring can achieve a host of biological actions, through subtle electron density changes in the substituent framework. Other factors including absorption, transport, and site of intracellular action might also be of importance.

This study also demonstrates that compounds **5a** and **5b** are NO-mediated vascular relaxation via aortic eNOS activation. This may lead to decreased BP in a hypertensive animal model. The 2 compounds exhibited excellent bioactivity profiles indicating that they might be multifunctional lead compounds for the purpose of improving

activity. Correlating the significant hypotensive and vasodilating activities of compounds **5a,b** bearing a hydrazine moiety at C2, led to the investigation of the mechanism of vasodilatation of other antihypertensive hydrazines. In particular, Hydralazine (Apresoline[®]), has been in clinical use as an anti-hypertensive agent for nearly 5 decades, but its mechanism of action remained poorly understood. It causes vasodilation as a result of a direct action on vascular smooth muscle cells at an intracellular site to cause vasorelaxation (Ellershaw and Gurney 2001). It was previously shown to be a directly acting vasodilator, evoking a slowly developing, long-lived relaxation in rabbit aorta rings. It causes vasodilation as a result of a direct action on vascular smooth muscle cells at an intracellular site to cause vasorelaxation (Ellershaw and Gurney 2001). It does not act as a calcium antagonist, because it is a poor inhibitor of vasoconstriction caused by elevated extracellular K^+ (Khayyal et al. 1981; Higashio and Kuroda 1988; Gurney and Allam 1995) and its effectiveness at inhibiting phenylephrine-induced contraction is little affected by removing extracellular Ca^{2+} (Higashio and Kuroda 1988; Gurney and Allam 1995; Orallo et al. 1991).

Conclusion

In summary, the present work is an extension of an ongoing effort toward the development and identification of new molecules with antihypertensive activity. In the present investigation, bioisosterism, and hybrid pharmacophore-based drug design led to the identification of multifunctional non-chiral C4-pyrimidine derivatives with antihypertensive potency. Compounds **5a**, **5b**, **9b**, **9c** exhibited promising lowering on MABP, calcium channel blocking activity, and decrease in heart rate. It could be concluded clearly that, synthesized pyrimidine derivatives containing C5-CN, C6-methyl (or phenyl) and C4-*p*-methoxyanilino moieties, showed variable activities. The impact of structural variation on activity was examined leading to that changes in C5-, C6-, and C4-positions could be important in receptor binding.

Compliance with ethical standards

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

Ethics approval All procedures performed involving animals were in accordance with the ethical standards. The experimental protocol was approved by Animal Care and Use Committee, Faculty of Pharmacy, Alexandria University (ACUC Project Number 16/1).

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