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Laboratory note

Synthesis and antiinflammatory activity of 4-amino-2-aryl-5-cyano-6-{3- and 4-(*N*-phthalimidophenyl)} pyrimidines[1]

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Abstract

Six new 4-amino-5-cyano-2,6-diarylpyrimidines **5a**–**h** has been synthesized in a facile manner by reacting the appropriate arylamidines **4a**–**d** with bisnitriles **3a**–**e**. Reduction of the nitro group of **5a**–**e** using Pd in ethyl acetate furnished **6a**–**e** in good yields. Reaction of **6a**–**e** individually with phthalic anhydride yielded **7a**–**e** in good to excellent yields. The newly synthesized heterocycles were characterized by IR, ^{1}H-NMR and mass spectral data. Compounds **5f**–**h** and **7a**–**e** were also evaluated against inflammation. Pyrimidines **5g**, **h** exhibited better antiinflammatory activity when compared with acetylsalicylic acid (ASA). Phthalimide derivatives **7a**–**e** also presented antiinflammatory activity, and three of them, viz., **7a**–**c** have been found to be twice more active than aspirin. Cytotoxical evaluations of compounds **7a**–**e** using neoplastic cells (NCI-H_{292} and Hep-2) presented 41% of growth inhibition of neoplastic cells NCI-H_{292}.

Keywords: Pyrimidines; Bisnitriles; Antiinflammatory activity

1. Introduction

Pyrimidine derivatives comprise a diverse and interesting group of drugs [1]. The subject has been discussed recently [2]. Earlier, a comprehensive review concerning pyrimidines has been published by Brown [3]. Pyrimidines in general are extremely important for their biological activities. For example, some are antiviral agents [4], the others are selective cholecystokinin subtype 1 (CCK1) receptor antagonists [5], and a few are antiinflammatory [6,7]. In fact, there are so many pyrimidine derivatives with pharmacological activities that it is difficult to describe much about them in the present paper.

In our continuing work on pyrimidines [8,9], we became interested to incorporate a phthalimido group in one of the phenyl rings. The reason for this is that phthalimide derivatives are gaining importance due to their different and significant biological activities [10]. Thalidomide is one of them, which was once prohibited in the market is again being considered important due to its activity as an antiinflammatory agent [10]. This drug has been found to reduce tumor growth by blocking angiogenesis both in model systems and in clinical studies [11–14]. Thalidomide and its analogs also slow down the replication rate of HIV-1 in vitro [15].

Since many phthalimide derivatives have presented hypolipidemic activity [16] and antiinflammatory activities [loc. cit.], we perceived that when two moieties, like phthalimide and pyrimidine are joined the molecules might exhibit superior antiinflammatory activity. It is with this idea in mind that the present work was undertaken. Therefore, this paper describes the synthesis of eight pyrimidine derivatives **5a**–**h** where six of them **5a**–**e**, **h** have not yet been reported in the literature. Compounds **5f**–**h** and **7a**–**e** have been tested for antiinflammatory property with impressive results. In fact, **7a**–**e** turned out to be very active against inflammation.

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2. Chemistry

The starting benzaldehyde and 3- and 4- substituted benzaldehydes **1a**–**e** were allowed to react with malononitrile **2** to give unsaturated bisnitriles **3a**–**e**. Condensation of these with arylamidines **4a**–**d** in the presence of piperidine furnished 4-amino-5-cyano-2,6-diarylpirimidines **5a′**–**h′** in good yields [8, 17]. Reduction of compounds **5a**–**e** using Pd/C provided their respective amines **6a**–**e**. Reaction of **6a**–**e** individually with phthalic anhydride yielded **7a**–**e** in good to excellent yields. It may be worthwhile to comment that only the amino group either at *meta* or *para* positions of the phenyl ring suffered selective reaction while the 4-amino group of the pyrimidine ring did not take part in the reaction at all. The formation of phthalimido-2-yl function at the *meta* and *para* positions in the C-6 phenyl ring of the substituted pyrimidines **7a**–**e** was verified by the ^{1}H-NMR, infrared (IR) and mass spectral data. The synthetic maneuvering leading to **7a**–**e** has been presented in Scheme 1.

3. Results and discussion

All substituted pyrimidines are crystalline and stable compounds with high melting points. These can be recrystallized and are homogeneous by thin layer chromatography.

3.1. Spectroscopy

The IR and mass spectra of all compounds agreed with the proposed structures. The series **5a′**–**h′** have the correct chemical shifts for the protons in the ^{1}H-NMR spectra and it was easy to characterize them. Similarly, the amines **6a**–**e** obtained by the reduction of the nitro groups presented the expected chemical shifts. The target compounds could also be characterized by ^{1}H-NMR without any problem and the chemical shifts of one of them, i.e. **7a** are given: H_2' and H_6' ($\delta = 8.43$ and $J = 9.0$ Hz); H3″ and H5″ ($\delta = 8.12$ and $J = 8.7$ Hz); δ 7.9 (b, 2H, NH_2) H_2″ and H_6″ ($\delta = 7.69$ and $J = 8.1$ Hz); H_3' and H_5' ($\delta = 7.61$ and $J = 8.4$Hz); H_1'″ and H_4'″ ($\delta = 8.02$ m); H_2'″ and H_3'″ ($\delta = 7.94$ m). δ 3.82 (s, 3H, CH_3O) the chemical shifts of all other compounds are given in Section 5.

4. Pharmacology

4.1. Preliminary toxicity and antiinflammatory tests of 4-amino-5-cyano-2,6-diarylpyrimidines *5f′–h′* and *7a–e*

The antiinflammatory assays were preceded by the acute toxicity tests in mice. The pyrimidine derivatives **5f′**–**h′** were dissolved individually in a 2.5% Tween 80 solution of 0.9% saline water. Administration of this suspension (0.5 ml) intraperitonially to the animals in doses of 125, 250, 500, 1000 mg kg^{-1} of body weight followed by the waiting period of 4–72 hours did not show any toxic effects. Pyrimidines containing phthalimido-2-yl group on the C-6 phenyl function in **7a**–**e** were also subjected to toxicity test which did not show any toxicity symptoms when evaluated in five groups each containing five animals under the same conditions described above. The doses were from 100, 200, 400, 800, 1600 mg kg^{-1} of the body weight.

Antiinflammatory tests have been performed for compounds **5f′**–**h′** and **7a**–**e** using groups of 10 Swiss albino mice at the dose of 250 mg kg^{-1}. All of them exhibited such property when compared with aspirin (acetylsalicylic acid, ASA) as well as indomethacin (INDO). Both of these commercially available antiinflammatory agents were also tested for comparative purposes employing the same solution as described above. Compound **5h′** showed the highest activity reducing the paw edema by 47.8%. Pyrimidines **5g′** and **5f′** presented 38.7% and 20.7% of inflammation reductions, respectively (Table 1).

Substances **7a**–**e** also presented antiinflammatory activity, but **7a**–**c** were much better in terms of inflammation reduction (Table 2). It is well known that INDO is stronger antiinflammatory drug than aspirin. A comparison of these two with compounds **7a**–**c** clearly demonstrate that compounds **7b** and **7c** are even superior in activity than INDO while **7a** is comparable to INDO (Tables 1 and 2). Figs. 1 and 2 provide the percentage reduction of edema for compounds **5f′**–**h′** and **7a**–**e** and their comparison with ASA and INDO.

Figs. 1 and 2 represent the comparative analyses of the antiinflammatory activity of compounds **5f′**–**h′** and **7a**–**e**.

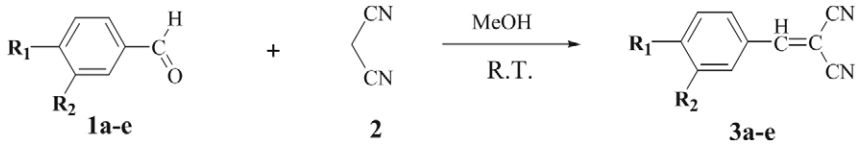
4.2. Cytotoxical test evaluations

Initially, compounds **5g′**–**h′** were tested for cytotoxical property but no significant activity was found. Next, phthalimidopyrimidines (**7a**–**e**) were tested, which did not present cytotoxical activity against HEp-2 in the concentrations tested. All compounds except **7e** inhibited the growth of the neoplastic cells NCI-H_{292}. The most active one was **7d** with 40.03% of inhibition activity at the concentration of 10 μg ml^{-1} (Table 3).

5. Experimental protocols

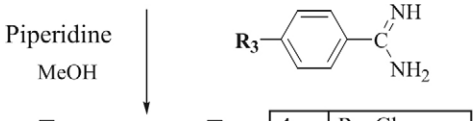
5.1. Chemical analysis

IR spectra were recorded with a Bruker spectrometer, model IFS 66 (Fourier Transform) utilizing KBr pellets. EI mass spectra were obtained with a Delsi-Nermag mass spectrometer, coupled to GC (HP 5890) at an ionization potential of 70 eV. ^{1}H-NMR spectra were recorded with a 300 MHz Varian spectrophotometer model UNITY plus. Melting points were obtained with an Electrothermal digital melting point apparatus (model 9100) and are uncorrected. Thin-layer chromatography was carried out on plates coated with silica gel containing fluorescent indicator F_{254} and the spots were detected under ultraviolet light. The solvent system was ethyl acetate/hexane (4:1) for compounds **5a**–**h** and ethyl acetate/hexane (3:1) for compounds **6a**–**e** and **7a**–**e**.



1a-e + 2 → 3a-e

1a and 3a	R_1=NO_2; R_2=H
1b and 3b	R_1=H; R_2= NO_2
1c and 3c	R_1=Cl; R_2=H
1d and 3d	R_1=OCH_3; R_2=H
1e and 3e	R_1=R_2=H

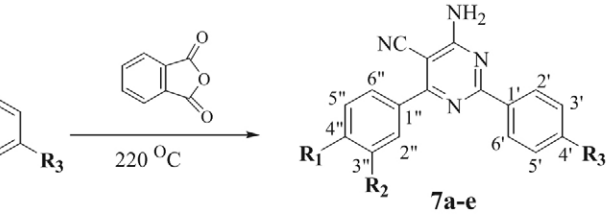


4a	R_3=Cl
4b	R_3=F
4c	R_3=OCH_3
4d	R_3=H

5a-h

5a'-h'

5a	R_1=NO_2; R_2=H and R_3= OCH_3
5b	R_1=NO_2; R_2=R_3=H
5c	R_1=NO_2; R_2=H and R_3= Cl
5d	R_1=NO_2; R_2=H and R_3=F
5e	R_1= H; R_2=NO_2 and R_3=Cl
5f	R_1=Cl; R_2=R_3= H
5g	R_1=R_2=R_3=H
5h	R_1=OCH_3; R_2=H and R_3=OCH_3



6a-e → **7a-e**

6a	R_1=NH_2; R_2=H and R_3=OCH_3
6b	R_1=NH_2; R_2=R_3=H
6c	R_1=NH_2; R_2=H and R_3=Cl
6d	R_1= NH_2; R_2=H and R_3=F
6e	R_1=H;R_2=NH_2 and R_3=Cl

7a	R_1=Phth; R_2=H and R_3=OCH_3
7b	R_1=Phth; R_2=R_3=H
7c	R_1=Phth; R_2=H and R_3=Cl
7d	R_1= Phth; R_2=H and R_3=F
7e	R_1=H; R_2=Phth and R_3=Cl

Phth =

Scheme 1.

Table 1
Antiinflammatory test results of compounds **5f**–**h**, ASA and INDO

Compound	Average difference in paw weights (g); standard deviation (S.D.)	Edema reduction (%)
2.5% Tween 80 in 0.9% aqueous NaCl	0.1045 ± 0.007	0.0
INDO	0.5081 ± 0.012	51.4
ASA	0.06680 ± 0.001	36.5
5f′	0.08280 ± 0.003	20.7
5g′	0.06432 ± 0.002	38.7
5h′	0.05450 ± 0.005	47.8

Table 2
Antiinflammatory test results of compounds **7a**–**e**

Compound	Average difference in paw weights (g); standard deviation (S.D.)	Edema reduction (%)
2.5% Tween 80 in 0.9%	0.16487 ± 0.005	0.0
Aqueous NaCl INDO	0.05081 ± 0.012	69.2
ASA	0.10436 ± 0.008	36.7
7a	0.08454 ± 0.010	65.7
7b	0.05986 ± 0.010	73.6
7c	0.06066 ± 0.007	73.2
7d	0.11122 ± 0.012115	32.53
7e	0.116120 ± 0.01494	29.54

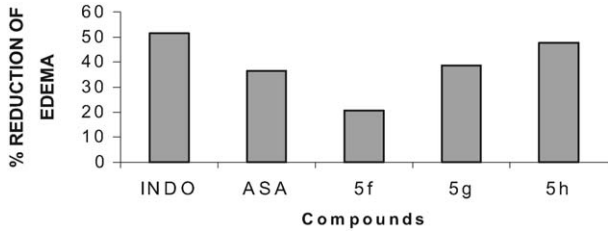


Fig. 1. Comparative analyses of the antiinflammatory activity of compounds **5f**–**h**, ASA and INDO.

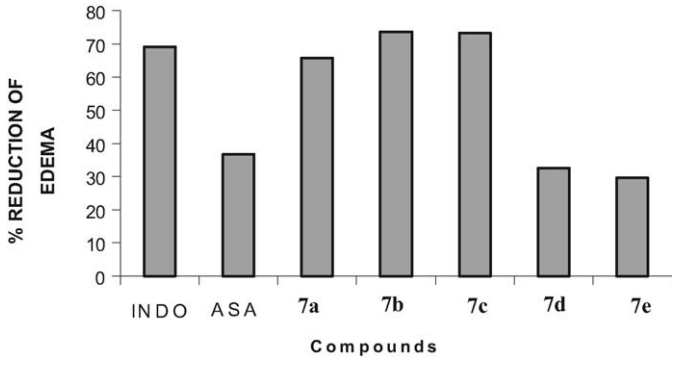


Fig. 2. Comparative analyses of the antiinflammatory activity of compounds **7a**–**e**, ASA and INDO.

5.2. General method for the preparation of bisnitriles (3)

An appropriate aldehyde **1** (24 mmol) and malononitrile **2** (24 mmol) were dissolved in methanol under stirring, and the contents stirred for 4 h at room temperature. The reaction was monitored by thin layer chromatography. Solvent evaporation left a solid mass, which was chromatographed over a silica gel column. Elution with n-hexane/ethyl acetate (4:1) afforded the desired compounds. The nitriles described in this work are known and their melting points agreed with the literature [18,19].

5.3. General method for the preparation of 4-amino-2,6-diarilpyrimidines-5-cyano (**5a**–**h**)

Bisnitrile **3** (5.36 mmol) and arylamidine **4** (5.36 mmol) were dissolved in methanol (20 ml) and refluxed for 7 h. The contents were cooled to room temperature and solvent evaporated under reduced pressure to give a solid mass, which was chromatographed over silica gel. The desired compound was eluted using a mixture of n-hexane/ethyl acetate (8:2). The fractions containing **5** were combined, solvent evaporated, and the product crystallized from methanol in every case.

5.3.1. 4-Amino-2-(p-anisyl)-5-cyano-6-(p-nitrophenyl)-pyrimidine (**5a**)

This compound was obtained as colorless crystals in 32% yield, m.p. 238–240 °C; $R_f = 0.41$ (n-hexane/ethyl acetate, 8:2); IR (KBr, γ_{max} cm^{-1}) 3446 (NH_2), 3356 (NH_2), 2209 (CN), 1648 (C=N); ^{1}H-NMR: (DMSO-d_6 300 MHz), δ 8.42 (d, 2H, $J = 8.1$Hz, H_2', H_6'), δ 8.48 (d, 2H, $J = 8.7$ Hz, H_3, H_5), δ 7.14 (l, 2H, NH_2), δ 8.25 (d, 2H, $J = 8.4$ Hz, H_2, H_6), δ 7.14 (d, 2H, $J = 8.1$ Hz, H_3', H_5'), δ 3.65 (s, 3H, CH_3O), MS: *m/z* (rel. int.) 347 (M^+, 100), 214 (35.5). Anal. calculated for $C_{18}H_{13}N_5O_2 \cdot 1/4H_2O$: C, 61.44%; H, 3.86%; N, 19.90%. Found: C, 61.40%; H, 3.75%; N, 19.96%.

5.3.2. 4-Amino-5-cyano-6-(p-nitrophenyl)-2-(phenyl)-pyrimidine (**5b**)

This compound was obtained as colorless crystals in 33% yield, m.p. 265–266 °C; $R_f = 0.45$ (n-hexane/ethyl acetate, 8:2); IR (KBr, γ_{max} cm^{-1}): 3421 ($NH_{2\ symm}$), 3339 ($NH_{2\ asymm}$), 2218 (CN), 1639 (C=N); ^{1}H-NMR (DMSO-d_6 300 MHz); δ 8.42 (2H, d, $J = 8.7$ Hz, H_2' and H_6'), δ 8.43 (2H, d, $J = 8.7$ Hz, H_3, H_5) δ 8.09 (2H, b, NH_2), δ 7.37 (3H), m, H_3', H_4' and H_5', δ 8.21 (2H, d, $J = 8.7$ Hz, H_2, H_6). MS: *m/z* (rel. int.) 317 (M^+, 100), 318 (49.66), 214 (25.9), 303 (12.41), 270 (10.44). Anal. calculated for $C_{17}H_{11}N_5O_2$: C, 64.35%; H, 3.49%; N, 22.07%. Found: C, 64.24%; H, 3.47%; N, 22.01%.

5.3.3. 4-Amino-2-(p-chlorophenyl)-5-cyano-6-(p-nitrophenyl)-pyrimidine (**5c′**)

This compound was obtained as colorless crystals in a yield of 55%; m.p. 319–320 °C; $R_f = 0.17$ (n-hexane/ethyl acetate, 8:2); (KBr, ν_{max} cm^{-1}): 3469 ($NH_{2\ asymm}$), 3346 ($NH_{2\ symm}$), 2220 (CN), 1645 (C=N), ^{1}H-NMR (DMSO-d_6 300 MHz), δ 8.42 (2H, d, $J = 8.7$ Hz, H_2', H_6'), δ 8.39 (2H, d, $J = 8.7$ Hz, H_3, H_5), δ 8.13 (2H, b, NH_2), δ 8.21 (2H, d, $J = 8.7$ Hz, H_2, H_6''), δ 7.61 (2H, d, $J = 8.7$ Hz, H_3', H_5'). Anal. calculated for $C_{17}H_{10}N_5O_2Cl \cdot 1/4H_2O$: C, 57.31%; H, 2.97%; N, 19.65%. Found: C, 57.54%; H, 2.67%; N, 19.73%.

Table 3
Cytotoxic activity in vitro of 4-amino-2-aryl-5-cyano-6-{3- and 4-(*N*-phthalimidophenyl)} pyrimidines **7a**–**e**

	Growth inhibition (%)			
	NCI-H_{292}			
Concentration (μg ml^{-1})	1.25	2.50	5.0	10.0
Compound **7a**	9.31 ± 0.023	16.55 ± 0.011	24.38 ± 0.042	26.69 ± 0.020
7b	7.09 ± 0.006	9.55 ± 0.032	10.55 ± 0.016	18.78 ± 0.029
7c	8.22 ± 0.016	17.30 ± 0.034	28.17 ± 0.035	30.97 ± 0.036
7d	8.07 ± 0.016	24.39 ± 0.034	27.51 ± 0.035	40.03 ± 0.036
7e	–	–	–	–

*5.3.4. 4-Amino-5-cyano-2-(p-fluorophenyl)-6-(p-nitrorophenyl)-pyrimidine (**5d′**)*

This compound was obtained as colorless crystals in a yield of 44%; m.p. 254–256 °C; $R_f = 0.57$ (n-hexane/ethyl acetate, 8:2); (KBr, γ_{max} cm^{-1}): 3417 ($NH_{2\ asymm}$), 3334 ($NH_{2\ symm}$), 2219 (CN), 1640 (C=N); ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 8.42 (4H, d, $J = 8.7$ Hz, H2′, H_6′, H_3□, H_5□), δ 8.16 (2H, b, NH_2), δ 7.36 (2H, t, H_3′, H_5′), δ 8.21 (2H, d, $J = 8.7$ Hz, H_2□, H_6□). Anal. calculated for $C_{17}H_{10}N_5O_2F{\cdot}1/4H_2O$: C, 60.59%; H, 3.00%; N, 20.88%. Found: C, 60.59%; H, 2.94%; N, 20.45%.

*5.3.5. 4-Amino-5-cyano-2-(p-chlorophenyl)-6-(m-nitrophenyl)-pyrimidine (**5e′**)*

This compound was obtained as colorless crystals in a yield of 53%; m.p. 295–296 °C; $R_f = 0.29$ (n-hexane/ethyl acetate, 8:2); (KBr, γ_{max} cm^{-1}): 3486 (NH_2′ $_{asymm}$), 3331 ($NH_{2\ symm}$), 2216 (CN), 1630 (C=N); ^{1}H-NMR (DMSO-d_6 300 MHz), δ 8.38 (2H, d, $J = 8.7$ Hz, H_2′, H_6′), δ 8.42 (1H, d, $J = 8.7$ Hz, H_2□), δ 8.39 (2H, b, NH_2), δ 7.62 (2H, d, $J = 8.4$ Hz, H_3′, H_5′), δ 8.76 (1H, t, $J = 1.8$ Hz, $J = 2.1$Hz, H_6□), δ 8.47 (1H, t, $J = 8.7$ Hz, $J = 1.8$ Hz, H_4□), δ 7.9 (1H, t, $J = 7.8$ Hz, $J = 8.1$ Hz, H_5□). Anal. calculated for $C_{17}H_{10}N_5O_2Cl{\cdot}1/4H_2O$: C, 57.47%; H, 2.97%; N, 19.71%. Found: C, 57.68%; H, 2.76%; N, 19.74%.

5f′ and 5g′: The melting points and spectroscopic data of these compounds agreed with the reported data in [17].

*5.3.6. 4-Amino-2-(p-anisyl)-6-(p-anisyl)-5-cyano-pyrimidine (**5h′**)*

This compound was obtained as colorless crystals in a yield of 84%; m.p. 191–192 °C; $R_f = 0.28$ (n-hexane/ethyl acetate, 8:2); (KBr, γ_{max} cm^{-1}): 3446 ($NH_{2\ asymm}$), 3356 ($NH_{2\ symm}$), 2209 (CN), 1648 (C=N); ^{1}H-NMR (DMSO-d_6, 500 MHz); δ 8.37 (2H, d, $J = 5.1$ Hz, H_2′, H_6′), δ 8.02 (2H, d, $J = 5.4$ Hz, H_2□, H_6□), δ 7.76 (2H, L, NH_2), δ 7.14 (2H, d, $J = 5.4$ Hz, H_3′, H_5′), δ 7.09 (2H, d, $J = 5.4$ Hz, H_3□, H_5□), δ 3.86 (6H, s, CH_3O), MS: *m/z* (rel. int.) 332 (M^+, 100), 331 (65.2), 199 (24.9). Anal. calculated for $C_{19}H_{16}N_4O_2{\cdot}1/4H_2O$ C, 68.66%; H, 4.85%; N, 16.86%. Found: C, 68.99%; H, 4.76%; N, 17.19%.

*5.4. General method for the preparation of compounds (**6a**–**e**)*

Nitro pyrimidines (**5a′**–**e′**) (5.36 mmol) were dissolved in ethyl acetate and 5% Pd on charcoal (20% by weight) was added to it. The contents were stirred at room temperature for 2 h under hydrogen atmosphere, and filtered to remove the catalyst followed by the solvent removal and crystallization from ethyl acetate.

*5.4.1. 4-Amino-6-(p-aminophenyl)-2-(p-anisyl)-5-cyano-pyrimidine (**6a**)*

This compound was obtained as yellow crystals in a yield of 90%; m.p. 177–178 °C; $R_f = 0.53$ (n-hexane/ethyl acetate, 8:2); (KBr, γ_{max} cm^{-1}): 3442 ($NH_{2\ asymm}$), 3344 ($NH_{2\ symm}$), 2203 (CN), 1610 (C=N); ^{1}H-NMR: (DMSO-d_6 300 MHz), δ 8.34 (d, 2H, $J = 9.0$ Hz, H_2′, H_6′), δ 7.85 (d, 2H, $J = 9.0$ Hz, H_2□, H_6□), δ 7.75 (b, 2H, C_4-NH_2), δ 7.04 (d, 2H, $J = 9.0$ Hz, H_3′, H_5′), δ 6.68 (d, 2H, $J = 8.7$ Hz, H_3□, H_5□), δ 5.65 (s, 2H, 4″-NH_2), the NH_2 signals disappeared after addition of D_2O; 3.65 (3H, s, CH_3O); MS: *m/z* (rel. int.) 347 (M^+, 100), 348 (24.31), 214 (36.43).

*5.4.2. 4-Amino-6-(p-aminophenyl)-5-cyano-2-(phenyl)-pyrimidine (**6b**)*

This compound was obtained as yellow crystals in a yield of 76%; m.p. 185–186 °C; $R_f = 0.27$ (n-hexane/ethyl acetate, 8:2); (KBr, ν_{max} cm^{-1}): 3323 ($NH_{2\ asymm}$), 3212 ($NH_{2\ symm}$), 2201 (CN), 1611 (C=N); ^{1}H-NMR: (DMSO-d_6, 300 MHz), δ 8.40 (dd, 2H, $J = 8.0$ Hz; $J = 2.0$ Hz, H_2′, H_6′), δ 7.90 (d, 2H, $J = 8.7$ Hz, H_2□, H_6□), δ 7.69 (b, 2H, 4″-NH_2), 7.53 (m, 3H, H_3′, H_4′, H_5′); 6.69 (d, 2H, $J = 8.7$Hz, H_3□, H_5□); δ 5.89 (s, 2H, C_6-NH_2), the NH_2 signals disappeared after addition of D_2O; MS: *m/z* (rel. int.) 317 (M^+, 100), 318 (49.66), 214 (25.89).

*5.4.3. 4-Amino-6-(p-aminophenyl)-5-cyano-2-(p-chlorophenyl)-pyrimidine (**6c**)*

This compound was obtained as yellow crystals in a yield of 55%; m.p. 240–245 °C; $R_f = 0.23$ (n-hexane/ethyl acetate, 8:2); (KBr, γ_{max} cm^{-1}): 3464 (NH_2), 3418 (NH_2), 2202 (CN), 1618 (C=N); ^{1}H-NMR: (DMSO-d_6, 300 MHz), δ 8.46 (d, 2H, $J = 9.0$ Hz; H_2′, H_6′), δ 7.95 (d, 2H, $J = 9.0$ Hz, H_2□, H_6□), δ 7.81 (b, 2H, C_4-NH_2), 7.66 (d, 2H, $J = 9.0$ Hz; H3′, H_5′); 6.75 (d, 2H, $J = 9.0$ Hz, H_3□, H_5□); δ 5.97 (b, 2H, 4″-NH_2), the NH_2 signals disappeared after the addition of D_2O.

*5.4.4. 4-Amino-6-(p-aminophenyl)-5-cyano-2-(p-fluorophenyl)-pyrimidine (**6d**)*

This compound was obtained as yellow crystals in a yield of 69%; m.p. 262–263 °C; $R_f = 0.22$ (n-hexane/ethyl acetate, 8:2); (KBr, γ_{max} cm^{-1}): 3465 (NH_2), 3412 (NH_2), 2203 (CN), 1605

(C=N); ^{1}H-NMR: (DMSO-d_6, 300 MHz), δ 8.44 (q, 2H, J = 8.7 Hz; J = 5.0 Hz, H_2', H_6'); 7.90 (d, 2H, J = 8.70 Hz, H_2, H_6); 7.65 (b, 2H, C_4-NH_2); 7.34 (m, 2″H, H_3', H_5'); 6.69 (d, 2H, J = 9.0 Hz, H_5, H_3), 5.85 (s, 2H, 4″-NH_2), the NH_2 signals disappeared after addition of D_2O).

5.4.5. 4-Amino-6-(m-aminophenyl)-5-cyano-2-(p-chlorophenyl)-pyrimidine (**6e**)

This compound was obtained as yellow crystals in a yield of 55%; m.p. 237–238 °C; R_f = 0.21 (n-hexane/ethyl acetate, 8:2); (KBr, γ_{max} cm^{-1}): 3423 (NH_2), 3342 (NH_2), 2208 (CN), 1650 (C=O); ^{1}H-NMR: (DMSO-d_6, 300 MHz), δ 8.38 (d, 2H, J = 9.0 Hz; H_2', H_6'), δ 7.13 (2H, m, H_2, H_5), δ 7.07 (d, 1H, J = 9.0 Hz, H_6), δ 7.88 (b, 2H, 4″-NH_2), 7.60 (d, 2H, J = 9.0 Hz, H_3', H_5'); 6.76 (d, 1H, J = 9.0 Hz, H_4), δ 5.35 (s, 2H, 4″-NH_2) the NH_2 signals disappeared after addition of D_2O.

5.5. General procedure for the synthesis of compounds (**7a–e**)

Compound **6** was dissolved in nitrobenzene with phthalic anhydride. The contents were refluxed and stirred for 30 min. The solid mass was precipitated using cyclohexane and the desired compound **7** was crystallized from acetic acid.

5.5.1. 4-Amino-2-(p-anisyl)-5-cyano-6-{4-(N-phthalimidophenyl)} pyrimidine (**7a**)

This compound was obtained in a yield of 67%; m.p. 335–340 °C; R_f = 0.60 (n-hexane/ethyl acetate 8:2); (KBr, γ_{max} cm^{-1}): 3459 (NH_2 asymm), 3361 (NH_2 symm), 2212 (CN), 1750 (C=N), 1644 (C=O); ^{1}H-NMR: (DMSO-d_6 300 MHz/δ ppm), δ 8.4 (2H, d, J = 9.0 Hz, H_2', H_6'), δ 8.11 (d, 2H, J = 8.4 Hz, H_3, H_5), δ 8,02 (m, 2H, H_1' H_4'), δ 7.9 (b, 2H, NH_2, after addition of D_2O the signal disappeared), δ 7.94 (m, 2H, H_2', H_3'), δ 7.68 (d, 2H, J = 8.4Hz, H_2, H_6), δ 7.08 (d, 2H, J = 8.7 Hz, H_3', H_5'), δ 3.82 (s, 3H, CH_3O); ^{13}C-NMR (DMSO-d_6): δ 167.41, 166.86, 164.46, 163.84, 162.23, 136.15, 134.85, 134.10, 131.60, 130.41, 129.20, 128.89, 127.22, 123.59, 116.56, 113.94, 83.75, 55.42; MS: *m*/*z* (int. rel.) 317 (M^+, 100), 403 (44.2), 316 (81.6). Anal. calculated for $C_{26}H_{17}N_5O_3$·1/2H_2O: C, 68.41%; H, 3.75%; N, 15.34%. Found: C, 68.62%; H, 3.52%; N, 15.30%.

5.5.2. 4-Amino-5-cyano-2-(phenyl)-6-{4-(N-phthalimidophenyl)}-pyrimidine (**7b**)

This compound was obtained in a yield of 64%; m.p. 327–328 °C; R_f = 0.29 (n-hexane/ethyl acetate 8:2); (KBr, γ_{max} cm^{-1}): 3404 (NH_2 asymm), 3355 (NH_2 symm), 2210 (CN), 1718 (C=N), 1609 (C=O), ^{1}H-NMR: (DMSO-d_6 300 MHz/δ ppm), δ 8.44 (2H, d, J = 7.5 Hz, H_2', H_6'), δ 8.13 (d, 2H, J = 8.7 Hz, H_3, H_5), δ 8.02 (m, 2H, H_1', H_4'), δ 7.9 (2H, b, NH_2, after addition of D_2O the signal disappeared), δ 7.94 (m, 2H, H_2', H_3'), δ 7.76 (2H, d, J = 8.4 Hz, H_2, H_6), δ 7.55 (d, 3H, J = 7.5 Hz, H_3', H_4', H_5'), ^{13}C-NMR (DMSO-d_6): δ 167.50, 166.85, 164.61, 164.10, 136.51, 136.01, 134.85, 134.18, 131.57, 129.88, 129.28, 128.58, 127.21, 123.59, 123.34, 116.45, 84.58; MS: *m*/*z* (int. rel.) 417 (M^+, 100), 418 (18.75), 314 (62.13). Anal. calculated for $C_{26}H_{17}N_5O_3$·1/2H_2O: C, 68.41%; H, 3.75%; N, 15.34%. Found: C, 68.62%; H, 3.52%; N, 15.30%.

5.5.3. 4-Amino-5-cyano-2-(4-chlorophenyl)-6-{4-(N-phthalimidophenyl)}pyrimidine (**7c**)

This compound was obtained in a yield of 61%; m.p. 312–313 °C; R_f = 0.26 (n-hexane/ethyl acetate 8:2); (KBr, γ_{max} cm^{-1}): 3471 (NH_2 asymm), 3445 (NH_2 symm), 2250 (CN), 1730 (C=N), 1611 (C=O); ^{1}H-NMR: (DMSO-d_6 300 MHz/δ ppm), δ 8.43 (d, 2H, J = 9.0 Hz, H_2', H_6'), δ 8.12 (d, 2H, J = 8.7 Hz, H_3, H_5), δ 8.02 (m, 2H, H_1', H_4'), δ 7.94 (2H, m, H_2', H_3'), δ 7.69 (d, 2H, J = 8.1 Hz, H_2, H_6), δ 7.61 (d, 2H, J = 8.4 Hz, H_3', H_5'); It was not possible to locate the -NH_2 protons ^{13}C-NMR (DMSO-d_6): δ 167.59, 166.86, 164.56, 163.08, 136.57, 135.86, 135.38, 134.88, 134.24, 131.59, 130.25, 129.28, 128.75, 127.23, 123.61, 116.32, 84.80. Anal. calculated for $C_{26}H_{17}N_5O_3$·1/2H_2O: C, 68.41%; H, 3.75%; N, 15.34%. Found: C, 68.62%; H, 3.52%; N, 15.30%.

5.5.4. 4-Amino-5-cyano-2-(4-fluorophenyl)-6-{4-(N-phthalimidophenyl)}pyrimidine (**7d**)

This compound was obtained in a yield of 68%; m.p. 314.5–315 °C; R_f = 0.28 (n-hexane/ethyl acetate 8:2); (KBr, γ_{max} cm^{-1}): 3466 (NH_2 asymm), 3317 (NH_2 symm), 2187 (CN), 1734 (C=N), 1644 (C=O); ^{1}H-NMR: (DMSO-d_6 300 MHz/δ ppm), δ 8.48 (d, 2H, J = 9.0 Hz, H_2', H_6'), δ 8.13 (d, 2H, J = 7.8 Hz, H_3, H_5), δ 8.0 (m, 2H, H_1', H_4'), δ 7.7 (d, 2H, J = 7.8 Hz, H_2, H_6), δ 7.94 (m, 2H, H_2', H_3'), δ 7.35 (t, 2H, J = 8.1 Hz, J = 8.4 Hz, H_3', H_5'); the -NH_2 protons presumably appeared 7.20 ppm ^{13}C-NMR (DMSO-d_6): δ 167.53, 166.85, 164.56, 163.11, 135.92, 135.34, 134.87, 134.21, 133.04, 131.56, 131.09, 129.89, 129.28, 127.21, 123.60, 116.38, 84.51. Anal. calculated for $C_{25}H_{14}N_5FO_2$·H_2O: C, 66.22%; H, 3.56%; N, 15.45%. Found: C, 66.22%; H, 3.31%; N, 15.21%.

5.5.5. 4-Amino-2-(4-chlorophenyl)-5-cyano-6-{3-(N-phthalimidophenyl)}pyrimidine (**7e**)

This compound was obtained in a yield of 50%; m.p. 309–310 °C; R_f = 0.34 (n-hexane/ethyl acetate 8:2); (KBr, γ_{max} cm^{-1}): 3460 (NH_2 asymm), 3351 (NH_2 symm), 2187 (CN), 1727 (C=N), 1642 (C=O); ^{1}H-NMR: (DMSO-d_6 300 MHz/δ ppm), δ 8.4 (d, 2H, J = 8.7 Hz, H_2', H_6'), δ 8.07 (m, 2H, H_4, H_6), δ 8.01 (m, 2H, H_1', H_4'), δ 7.93 (m, 2H, H_2', H_3'), δ 7.74 (d, 1H, J = 8.4 Hz, H_2), δ 7.72 (bs, 1H, H_5), δ 7.59 (2H, J = 8.4 Hz, H_3', H_5'); The -NH_2 protons could not be located ^{13}C-NMR (DMSO-d_6): δ 173.06, 167.36, 167.10, 164.71, 163.24, 137.27, 136.74, 135.40, 134.99, 132.46, 131.67, 130.35, 130.04, 129.25, 128.81, 128.33, 127.69, 123.69, 116.21. Anal. calculated for $C_{25}H_{14}N_5C_lO_2$·1/2H_2O: C, 65.15%; H, 3.28%; N, 15.26%. Found: C, 64.86%; H, 3.33%; N, 15.61%.

5.6. The antiinflammatory activity of the compounds **5** and **7**

The antiinflammatory activity was evaluated by the model of carrageenan-induced mouse paw edema following the literature method [20], which was described earlier by Levy [21]. The inflammation was induced by injecting a 1% solution of carrageenan lambda (sigma) dissolved in a 0.5% saline solution containing 2.5% of Tween 80, in the right hind paw of a group of 10 animals (body weight ≈ 19–21 g of each animal). After 30 min the compounds were administered to a group of 10 mice intraperitoneally having a dose of 250 mg kg^{-1}. The antiinflammatory drug used as standard was ASA at the dose of 250 mg kg^{-1}. Even with the discovery of many new medicines, aspirin is still the analgesic–antipyretic and antiinflammatory agent more prescribed in the medicine and is standard to the evaluation of new drugs in pharmacological assays [22].

The cytotoxical assays were performed using the methodology described by Mosman [23] to evaluate the cell proliferation. The drugs were dissolved in DMSO having four concentrations of 10, 5, 2.5 and 1.25 μl. A suspension at the concentration of 5×10^4 cells ml^{-1} were prepared and distributed in culture cell plates and incubated at 37 °C over a damp atmosphere. After 24 h the test drugs were added. DMSO was used as control and the test were made in quadruplicate. The cells were incubated for additional 72 h. After this time, a solution of 25 μl of MTT at a concentration of 5 mg ml^{-1} [24] was added. The plates were incubated at 37 °C for more than 2 h and the reading were made by an ELISA reader model ELX 800 at 540 nm.

6. Conclusion

We have achieved an efficient and simple synthesis of **5a**–**h**. The transformation of these to **6a**–**e** followed by the preparation of phthalimidoyl pyrimidines **7a**–**e** has also been accomplished. The discovery that these final phthalimidopyrimidines are antiinflammatory without any toxic effects is exciting. This finding is quite satisfactory. Hence, this study has widened the scope for evolving the new and promising antiinflammatory drugs.

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