Scientific paper

New Fused Pyrimidines of Potential Biosignificant Interest. Syntheses and Molecular Modelling Studies

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Abstract

New derivatives of thieno[3,2-*d*]pyrimidine and thieno[2,3-*b*]pyrrole **5a**,**b** and **6a**,**b**, respectively, were obtained from the corresponding thiophene-2-carboxamides **4a**,**b**. On the basis of compounds **5b**, **6a** and **6b**, two novel series of tricyclic- and tetracyclic-condensed pyrimidines **8**–**15** and **16**–**19**, respectively, were synthesized by the application of the cyclization reactions of **5b** and **6a**,**b** with a variety of commercially available reactants. Geometry optimization of selected structures, using the AM1 semiemperical method, revealed a smaller ionization potential and a lower degree of conformational freedom for the tetracyclic pyrimidine derivatives relative to their tricyclic counterparts. Interestingly, computation of the solvation free energies of the lowest energy conformers at physiological conditions indicated that the series is highly soluble under these conditions. The trend in solubility as implied by the relative magnitudes of the solvation free energies is suggestive of a greater contribution of higher moments of charge distribution in modulating the interaction of the structures with the biological environment which could be detrimental for the binding modes of these structures to their putative receptor sites.

Keywords: Methylation, carboxamides, pyrimidines, molecular modelling, AM1 calculations

1. Introduction

Nitrogen-containing heterocyclic compounds have displayed a broad spectrum of biological effects. Within the synthesis of heterocyclic *N*-containing compounds, pyrimidine and its derivatives attracted considerable attention as they are often present in biologically active compounds and many examples of biological activities found for small molecules based on pyrimidine moiety can be referred. Most importantly, they are of great importance in fundamental metabolism for uracil, thiamine and cytosine, that are three bases found in the nucleotide and hence pyrimidine bases play significant role in vital biochemical processes for humans and animals.[1,2] Amino substituted compounds take particular place in such processes.[1] The biological activity of some isolated alkaloids has been attributed to the presence of the dihydropyrimidinone moiety in the molecules[3,4] and the conformation of the pyrimidine ring.[3,5,6] Other derivatives of dihydropyrimidine (DHPM) have interesting biological properties such as antimicrobial,[7] antiviral[8] and anticancer[9] activities and moreover were found to be useful in the treatment of benign prostatic hyperplasia.[10] More recently, these partly reduced pyrimidine derivatives (DHPMs) have emerged as anti-inflammatory agents.[11] Very recently, *S*-alkylpyrimidines possessing antifungal and antibacterial activities have been also reported in the literature.[12] Furthermore, literature survey shows that amino- and imino- forms of pyrimidine are widely presented as part of antibiotics, corrective medications for heart failures and metabolic stimulators.[1] A very recent highlight in this context has been the identification of some derivatives of 2-phenylaminopyrimidine as potent inhibitors of spleen tyrosine kinase (SYK) and potential new lead for the treatment of asthma and allergic disorders.[13] In addition, numerous nucleosides containing 1-substituted pyrimidines have found utility as anticancer and antiviral chemotherapeutic

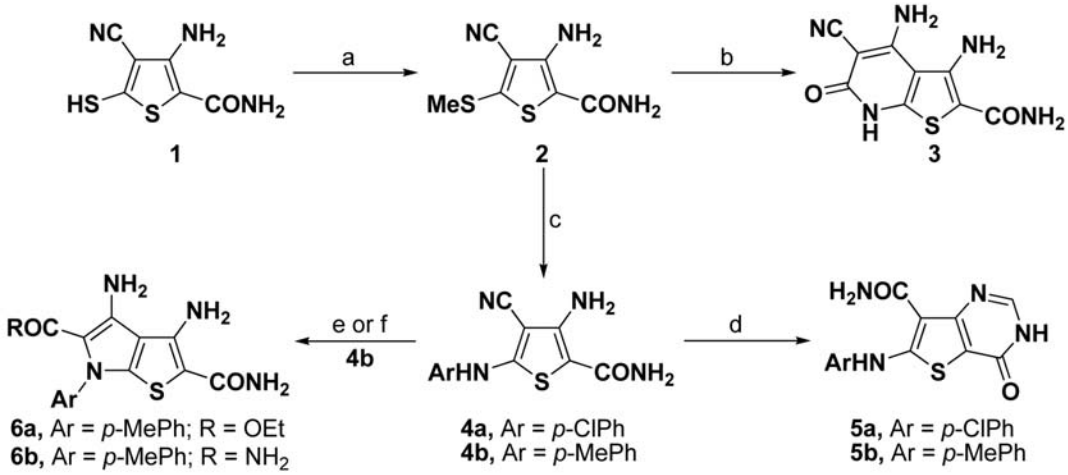
agents.[8,14,15] More specifically, 5-(2,3,5-trichlorophenyl)-2,4-diaminopyrimidine (BW1003C87) was reported some time ago as anticonvulsant as well as neuroprotective in models of brain ischaemia and white matter ischaemia.[16,17] In recent related studies,[18,19] *R*-(–)-2,4-diamino-6-(fluoromethyl)-5-(2,3,5-trichlorophenyl)-pyrimidine (BW202W92), a molecule which has displayed a unique voltage-gated sodium channel binding activity, and its *S*-enantiomer BW203W92 have been published. Within the class of nitrogen-containing heterocycles, pyrrole and its derivatives are ubiquitous in nature. As such, the pyrrole subunit has found widespread applications in therapeutically active compounds, including fungicides, antibiotics,[20–23] nonsteriodal anti-inflammatory drugs (NSAIDS),[24–26] cholesterol-reducing drugs[27] and anti-tumor agents.[28,29] On the other hand, literature survey reveals that thiophene-containing substances are also well known for their diverse pharmaceutical activities.[30] These include anticancer,[31–33] antifungal,[34] antimicrobial,[35–38] antifeedant and termiticidal,[39] antidepressant[40] and analgesic[40] effects. Thiophene ring system is also a structural part of the commercial imidazole antifungal agent sertaconazole,[41] of the anti-asthma drug zileuton,[42] of the non-nucleoside hepatitis B virus inhibitors (HBV)[43] and of the Na^+/H^+ exchanger isoform-1 (NHE-1) inhibitors.[44]

Given these reports, and the expectation of further applications based on the above-mentioned heterocyclic rings, it therefore seemed of practical interest to prepare substances containing pyrimidine annelated with thiophene ring in one frame of tricyclic-condensed system **8**–**15** and with both thiophene and pyrrole rings together in another tetracyclic frame **16**–**19**. Our interest in the synthesis of such compounds was to expand our investigations on condensed heterocycles as part of our medicinal program aimed at the development of new heterocyclic compounds that could have therapeutic and biological potential.[45–47]

2. Results and Discussion

Methylation of 5-mercaptothiophene derivative **1**[48] with methyl iodide produced the corresponding *S*-methylated product **2**. This synthetic route constitutes a new, facile and unambiguous synthesis of this versatile compound **2** rather than following the *Corsaro* procedure,[49] where the same compound was obtained through the sun light induced conversion of the corresponding 4-amino-3-(methylthio)thieno[2,3-*c*]isothiazole-5-carboxamide. A further reaction of 5-methylthio derivative **2** with cyanoacetamide gave the thienopyridine derivative **3**, while nucleophilic substitution by *p*-chloroaniline or *p*-toluidine led to the corresponding 5-arylamino derivatives **4a**,**b**. Ring closure of compounds **4a**,**b** with formic acid resulted in the formation of 6-arylamino-4-oxothieno[3,2-*d*]pyrimidine-7-carboxamides **5a**,**b**. The IR spectra of compounds **5a**,**b** contained no signals from the CN groups, but did contain characteristic signals of the CO amide and CO ring groups. The ^{1}H NMR of these compounds showed signals corresponding to the hydrogen attached to C-2 of the pyrimidine ring. Additionally, the signals from the 3-H proton of **5a** and **5b** were also observed at δ 12.11 and 12.53 ppm, respectively. Signals from the $CONH_2$ and Ar-NH moieties have been also detected in their proper positions. When compounds **4a**,**b** were allowed to react with each of ethyl chloroacetate and chloroacetamide, they afforded the corresponding 3,4-diaminothieno[2,3-*b*]pyrroles **6a**,**b**.

The bicyclic thiophenes **5b** and **6a**,**b** have been employed as synthetic foundations for the target tri- and tetra-cyclic pyrimidine systems (Schemes 2 and 3). Thus, cyclization of **5b** with phenyl isothiocyanate was carried out to yield a tricyclic product with annelated pyrimidine rings for which three isomeric structures can be formulated. However, the structure of isolated product was considered to be thione structure **8** rather than other potential



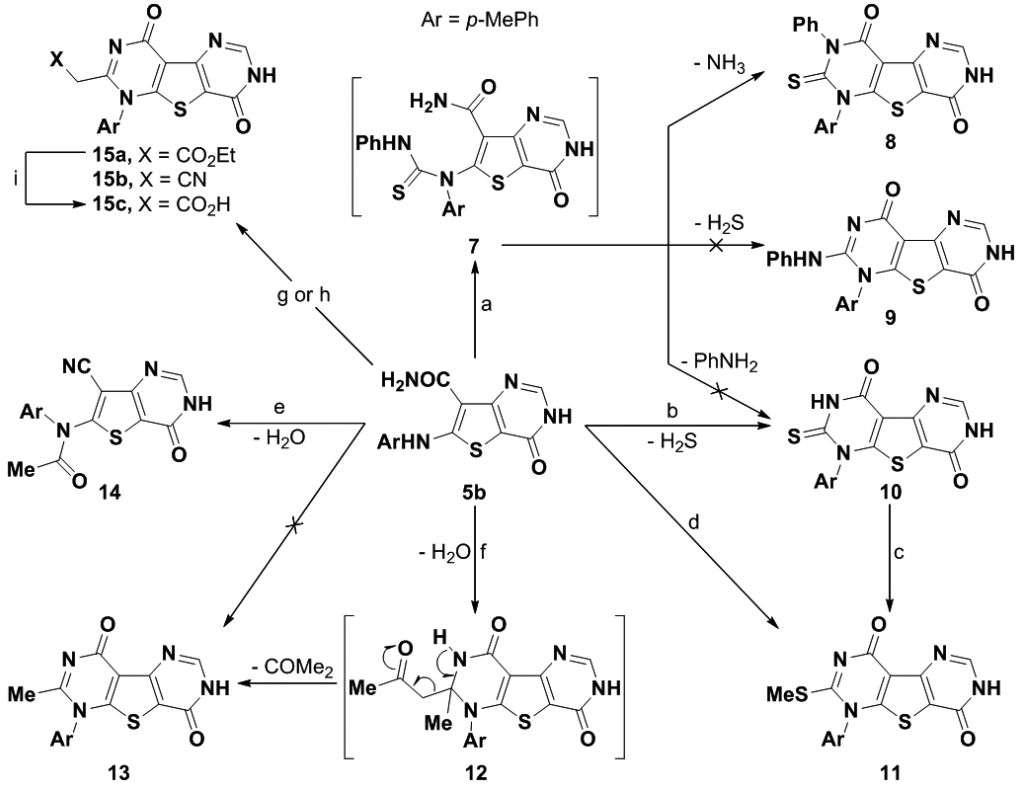
Reagents and conditions: (a) MeI/NaOEt/Δ; (b) $NCCH_2CONH_2$/py/Δ; (c) $ArNH_2$/fusion; (d) HCO_2H/Δ; (e) $ClCH_2CO_2Et/K_2CO_3$/DMF/Δ; (f) $ClCH_2CONH_2/K_2CO_3$/DMF/Δ

Scheme 1

isomeric structures **9** and **10** based on analytical and spectral data, besides a chemical proof. The IR and ^{1}H NMR spectra of the isolated product were informative in establishing the structure of thione derivative **8**. Thus, its IR spectrum has absorption bands at 1691 and 1672 cm^{-1} corresponding to stretching vibrations of the two CO groups, besides absorption band for the NH stretching vibration at 3150 cm^{-1}. Signals for all protons are present in its ^{1}H NMR spectrum (see Experimental section). In support of this view, the other possible regioisomer **10** was also excluded based on the non-identity of reaction product, obtained by reacting **5b** with phenyl isothiocyanate, with a sample of **10** prepared by the application of the cyclization reaction of **5b** with carbon disulfide in refluxing pyridine.

Nevertheless, a proof of structure **10** was accomplished by treating **10** with methyl iodide in refluxing ethanolic sodium ethoxide solution to provide the anticipated 7-methylthio derivative **11**, which was also obtained directly by reacting **5b** with carbon disulfide in the presence of potassium hydroxide in dimethylformamide (DMF) followed by treatment with methyl iodide (Scheme 2). This result is in agreement with a report in the literature[50] on a similar observation.

It is remarkable to report here that an unexpected reaction took place on reacting **5b** with acetic anhydride in an attempt to obtain the tricyclic derivative **13**. To our surprise, this reaction did not give the desired **13** and instead the *N*-*p*-tolylacetamide derivative **14** was isolated as indicated from the spectral data of the reaction product, where the IR spectrum of this product indicated the presence of CN group stretching at 2216 cm^{-1} and two CO groups stretching at 1677 and 1670 cm^{-1}, thus confirming the open-chain structure of **14** rather than the alternative structure **13**. Additionally, the mass spectrum was indicative of dehydrative acetylation product which showed a molecular ion peak M$^+$ at *m/z* 324. This was compatible with molecular formula $C_{16}H_{12}N_4O_2S$ that completely fits to the proposed structure **14**. In support of this view, the desired target **13** was obtained by using an alternative synthetic route involving the condensation of **5b** with acetylacetone in refluxing ethanol in the presence of a catalytic amount of concentrated hydrochloric acid. This result can be explained by assuming the formation of condensation product **12** as a first step. Subsequent aromatization *via* loss of acetone leads eventually to the final 7-methyl derivative **13**. Disappearance of two D_2O-exchangeable signals corresponding to enaminoamide moiety as well as appearance of a methyl singlet in the ^{1}H NMR of the isolated product proved that the amino and the carboxamido groups were both involved in the dehydrative cyclization step leading to **13**. The above result finds a great support by the previous reports in the recent literature concerning the same behaviour.[51–53]



Reagents and conditions: (a) PhNCS/NaOEt/Δ; (b) CS_2/py/Δ; (c) MeI/NaOEt/Δ; (d) CS_2/KOH/DMF/Δ then MeI/Δ; (e) Ac_2O/Δ; (f) Ac_2CH_2/EtOH/HCl/Δ; (g) $CH_2(CO_2Et)_2$/Δ; (h) $NCCH_2CO_2Et$/NaOEt/Δ; (i) KOH/ab EtOH/Δ then AcOH

Scheme 2

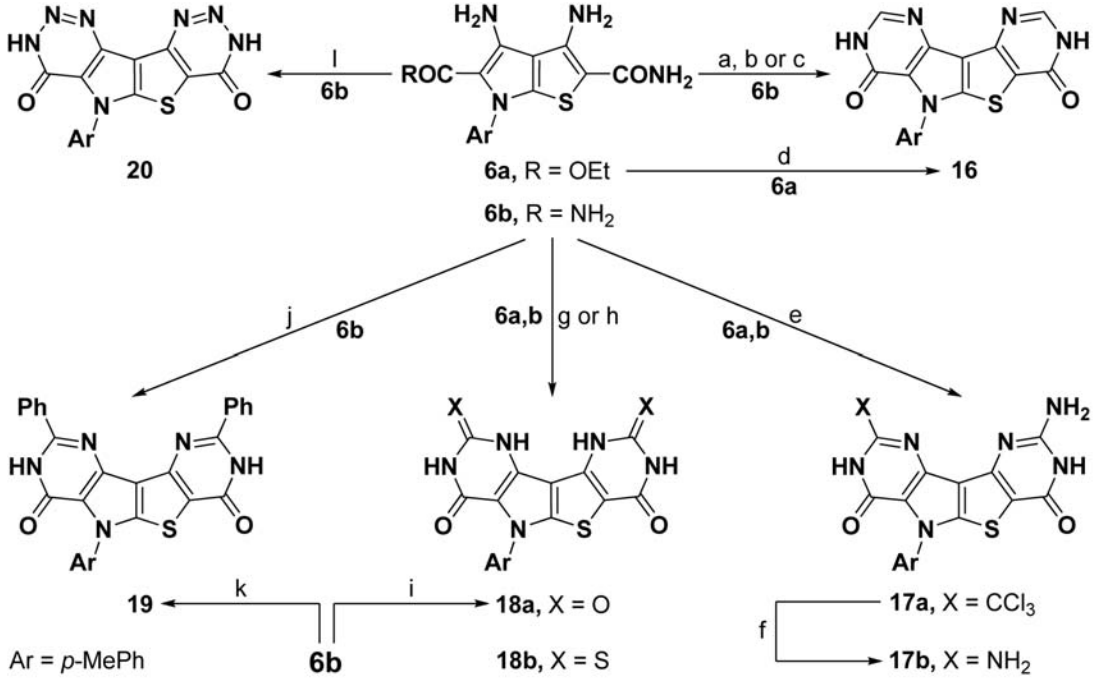
Reaction of **5b** with an excess of diethyl malonate at reflux led to the ethyl ester **15a**, while with ethyl cyanoacetate, the 7-cyanomethyl derivative **15b** was isolated in an acceptable yield. Assignment of the proposed structure **15a**,**b** for cyclization products was based on analytical and spectral data and confirmed by chemical transformation. Thus, saponification of the ethyl ester **15a** with potassium hydroxide in absolute ethanol followed by treatment with acetic acid yielded 7-acetic acid derivative **15c**.

The synthesis of the title tetracyclic pyrimidine derivatives has been accomplished from two thienopyrrole intermediates **6a** and **6b** (Scheme 3). Closure of the two pyrimidine rings of the pyrimidothienopyrimidopyrrole ring system was carried out by the application of the nucleophilic cyclization reactions of dicarboxamide analogue **6b** with different C-1 building blocks, namely; triethyl orthoformate, dimethylformamide dimethylacetal (DMFDMA) or formic acid, to produce in every case a single product for which the tetracyclic-condensed structure **16** was established on the basis of its analytical and spectral data. It is worth mentioning that the same product **16** could be also obtained by refluxing the ester analogue **6a** in formamide (Scheme 3). As a criterion of the cyclization of carboxamide **6b** or its carboxylic ester analogue **6a** into dione derivative **16** can serve the disappearance of the resonance signals from protons of the four NH_2 units or from protons of the ethyl and three NH_2 units, characteristic to compounds **6a**,**b**, and the appearance of only two D_2O-exchangeable singlets for the NH protons as well as other two singlets for the CH protons of the pyrimidine rings in the ^1H NMR spectrum of compound **16**.

Compounds **6a**,**b** could also be cyclized with trichloroacetonitrile to yield other tetracyclic pyrimidines **17a**,**b**. Another synthesis of **17b** involved the aminolysis of trichloromethyl compound **17a** in refluxing methanol in the presence of ammonium hydroxide solution led to its conversion into the diamino analogue **17b**. The reactivity of compounds **6a**,**b** towards urea and thiourea was also investigated as a possible route for the synthesis of target molecules. Accordingly, fusion of **6a** or **6b** with urea gave, in each case, a single product that was identified as **18a**. A third route for the synthesis of **18a** involved the treatment of dicarboxamide analogue **6b** with ethyl chloroformate. In the same way, the reaction of **6a** and **6b** with thiourea occurs with formation of dithione derivative **18b**. Elucidation of the proposed structure of the latter products was established on the basis of elemental analyses and spectral background in each case.

Benzoylation of dicarboxamide analogue **6b** with benzoyl chloride provided the respective 2,9-diphenyl derivative **19**. The structure of **19** was supported by an independent synthesis of the same product from **6b** and benzaldehyde in refluxing ethanol in the presence of a catalytic amount of concentrated hydrochloric acid (Scheme 3).

As an extension of such a synthetic route, we further explored the behaviour of the dicarboxamide analogue **6b** towards nitrous acid was also investigated as a possible route for the synthesis of triazine ring system. Thus, we



Reagents and conditions: (a) $CH(OEt)_3/Ac_2O/\Delta$; (b) $(MeO)_2CHNMe_2/DMF/\Delta$; (c) HCO_2H/Δ; (d) $HCONH_2/\Delta$; (e) $CCl_3CN/PhH/\Delta$; (f) NH_4OH MeOH/Δ; (g) $(NH_2)_2CO$/fusion; (h) $(NH_2)_2CS$/fusion; (i) $ClCO_2Et/py/\Delta$; (j) $PhCOCl/\Delta$; (k) PhCHO/EtOH/HCl/Δ; (l) AcOH/HCl/$NaNO_2$/0-5 °C

Scheme 3

applied the diazotization and self condensation of **6b** with sodium nitrite to the synthesis of tetracyclic triazindione derivative **20** (Scheme 3).

3. Molecular Modelling

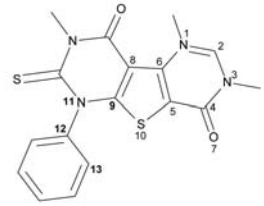
In order to investigate the effect of the different substitution schemes outlined in the previous sections on the geometrical and electrostatic properties of the synthesized pyrimidine derivatives, the putative geometries of theses structures were subjected to geometry optimization using the AM1 model.[54] Notably, the structures comprise a common moiety that encompasses a highly rigid fused ring system and a rotatable phenyl ring whose orientation is determined by the –N11–C12– dihedral angle (Table 1 and Figure 1). Inspection of the rotation profile of the –N11–C12– dihedral indicates that the structures can be broadly divided into two categories based on the modality of the phenyl ring energy profiles. The first of these categories comprises the **8**, **10**, **11**, **13**, **15a**, **15b** and **15c** structures whilst the second spans the rest of the series. Such categorization is not only commensurate with broadly dividing the series to tri- and tetra-cyclic pyrimidine derivatives but it also highlights their varying conformational freedom.

The rotation profiles in both categories are nearly symmetric due to the planar fused-ring system common to these structures and the similar chemical environments on either side of the phenyl ring. Noticeably, the rotation around the N11–C12 bond is highly restricted due to the relatively large energy barrier, ~ 4–10 kcal mol^{-1}, encountered at ~ 180^0 rotation (Figure 1) where the phenyl ring is almost coplanar with the fused-ring system.

Inspection of the rotation profile of the –N11–C12– dihedral of the first category; **8**, **10**, **11**, **13**, **15a**, **15b** and **15c**, indicates that the structures span a nearly two-minimum energy profile. The two minima correspond to structures that are related by 180^0 rotation of the phenyl ring and thereby correspond to nearly the same structure. The two energy minima correspond to structures where the phenyl ring is almost perpendicular, 80.0 to 90.0^0, to the plane of the fused rings (Table 1). This orientation relieves steric interaction and allows for circulation of the electronic cloud throughout the molecular skeleton (*e.g.* structure **8**, Figure 2).

Interestingly, in the second category; structures **16**, **17a**, **17b**, **18a** and **18b**, a small energy barrier is introduced within the 80.0 to 90.0^0 range forcing the phenyl ring to a tilted position relative to the plane of the fused ring system (*e.g.* structure **17b**, Figure 2). These structures differ from those of the first category primarily in the existence of a fourth fused ring. Formation of this ring immobilizes a carbonyl group that is prone to strong interaction with the phenyl hydrogens. The asymmetric interaction of the phenyl hydrogens with that carbonyl group from one side and with the sulphur atom from the other side creates the small energy barriers ~ 1.0 kcal mol^{-1} observed in the –N11–C12– dihedral profile for this category (structure **17b**, Figure 1).

Table 1. The conformational parameters and energetics of the lowest energy conformers of the tri- and tetra-cyclic pyrimidine derivatives. For the sake of symplicity, the tricyclic ring system common to both tri- and tetra-cyclic derivatives is only shown.



Structure	Dihedral C9-N11-C12-C13	ΔG_{solv} [kcal/mol]	Dipole Moment [Debye]	IP[a] [Hartree]	HOMO-LUMO gap [Hartree]
8	91.0	–41.051	2.951	0.317	0.271
10	89.3	–38.224	4.345	0.329	0.280
11	87.0	–39.132	5.399	0.326	0.292
13	88.1	–37.851	6.586	0.326	0.292
15a	81.3	–38.305	5.765	0.328	0.292
15b	88.9	–38.716	8.326	0.332	0.292
15c	91.0	–40.943	6.589	0.330	0.292
16	119.5	–39.255	1.580	0.301	0.270
17a	115.4	–36.367	1.479	0.298	0.261
17b	142.2	–26.517	4.438	0.288	0.263
18a	117.8	–39.671	4.184	0.314	0.276
18b	116.1	–40.865	6.251	0.317	0.267

[a] IP: The Ionization Potential

Inspection of the HOMO-LUMO energy gap and the ionization potential of the two categories (Table 1) reveal that the second category, structures **16**, **17a**, **17b**, **18a** and **18b**, span smaller ionization potentials and HOMO-LUMO energy gaps relative to the rest of the series. This is a direct consequence of the higher order of conjugation prevalent in these structures relative to those in the first category. Such reduction in ionization potential and HOMO-LUMO energy gap indicates that the **16**, **17a**, **17b**, **18a** and **18b** structures are more liable to electron transfer reactions which could have implications on their biological activity.

Given that the biological system is the native environment for *in vivo* studies of putative drug candidates,

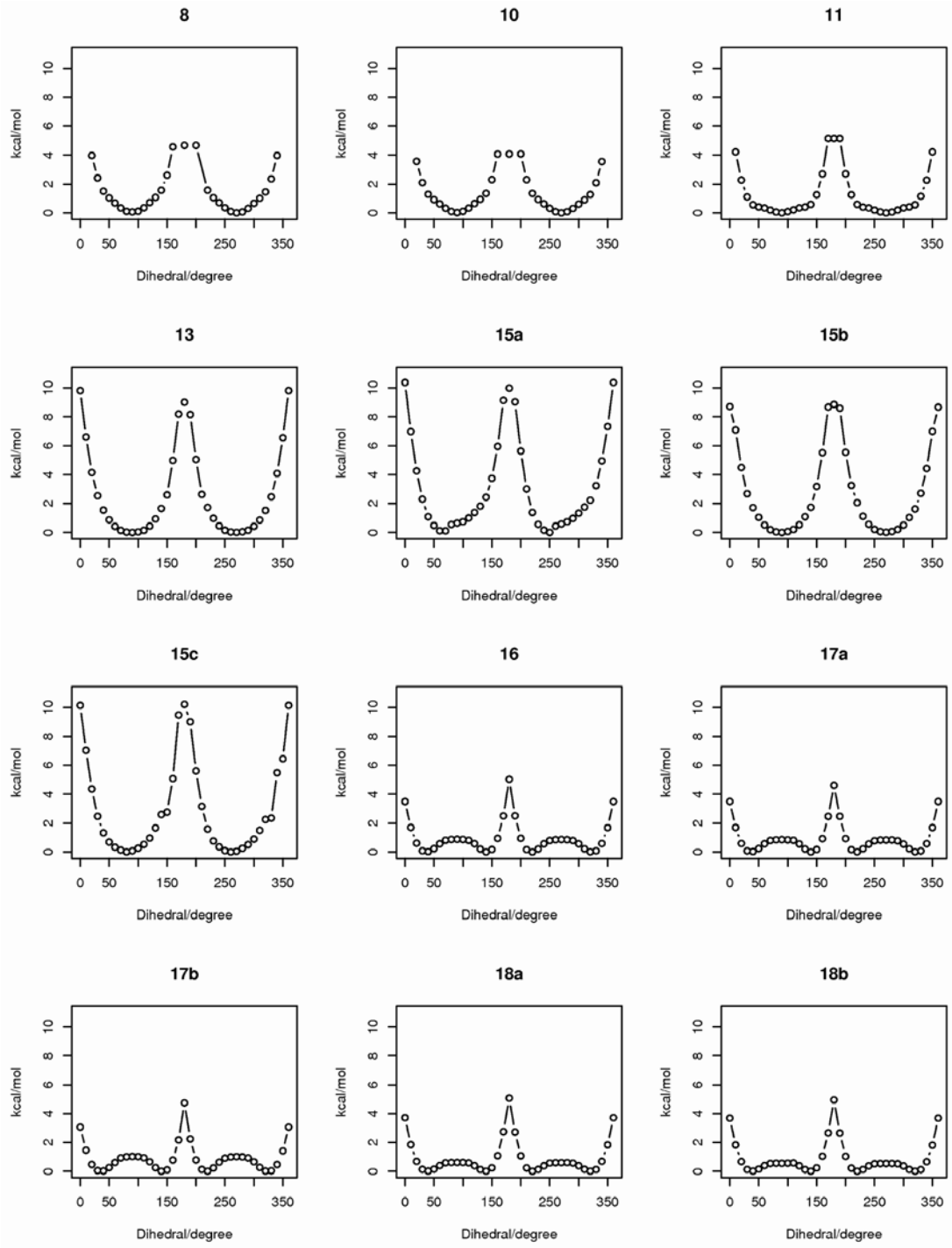


Figure 1. Energy profiles of the –N11–C12– dihedral rotation for selected tri- and tetra-cyclic pyrimidine derivatives. The energy profiles were obtained *via* applying constraints at incremental dihedral steps of 10 degrees and relaxation of the free degrees of freedom by geometry optimization using the AM1 semiemperical model.

the calculations have therefore been supplemented by computation of the solvation free energy (ΔG_{solv}) of some of the series members *via* solving the Poisson-Boltzmann equation[55] at physiological conditions (0.15 M NaCl and 310 K). Inspection of Table 1 reveals that the whole series shows favourable free energies of solvation that are within the range from –26 to –41 kcal mol^{-1} with the **17b** structure being the least stabilized. Such a favourable solvation free energy is indicative of the high solubility of these structures at physiological conditions. The high solubility can be attributed in part to the polarity of these structures as indicated by the large dipole moment of most of them (Table 1). However, the trend in solubility as implied by the relative magnitudes of the solvation free energies is not straightforwardly related to the relative magnitudes of the dipole moments. This is suggestive of a greater contribution of higher moments of charge distribution in modulating the interaction of these structures with the biological environment. Higher moments of charge distribution could therefore be detrimental for the binding modes of these structures to their putative receptor sites which is the subject of an ongoing study.

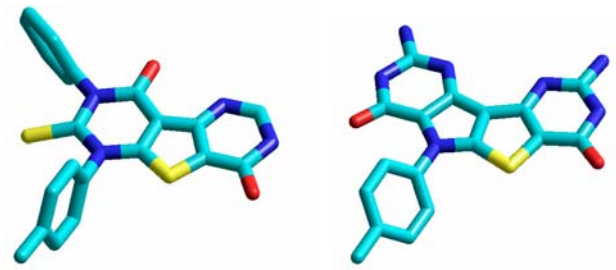


Figure 2. Optimized geometries of structure **8** (left) and **17b** (right). Structures correspond to the lowest energy minima of the –N11–C12– dihedral profiles shown in Figure (1).

4. Conclusion

The current study demonstrates the scope for the utility of carboxamides **5a** and **6a**,**b** in the construction of tricyclic- and tetracyclic-condensed heterocyclic systems by various chemical reagents. The biological potential of those systems was further investigated by molecular modelling techniques namely geometry optimization and calculation of the solvation free energies at physiological conditions. Analysis of the energy profiles of the tri- and tetra-cyclic structures revealed a varying degree of conformational flexibility of the two systems. This was found to be a direct consequence of immobilizion of a carbonyl group upon formation of the tetracyclic systems which creates a small energy barrier, 1–2 kcal mol^{-1}, and forces the phenyl ring to a tilted position relative to its counterparts in the tricyclic structures. On the other hand, the lowest energy conformers of the tri- and tetra-cyclic structures span a range of favourable solvation energies at physiological conditions with structure **8** being the most stabilized whilst structure **17b** is the least stabilized. The favourable solvation energies at physiological conditions are detrimental for the biological potential of the synthesized structures. Further studies on the biomedical applications of those condensed derivatives as chemotherapeutic agents are currently underway. We believe that intensive research in this direction should be encouraged and the results of this research will be reported in due course.

5. Experimental

All melting points are uncorrected. IR spectra (KBr) were recorded on a Pye Unicam SP-1000 spectrophotometer. NMR spectra were obtained on a Varian Gemini 300 MHz spectrometer in DMSO-d_6 as solvent and TMS as internal reference. Chemical shifts are expressed in δ ppm. EI mass spectra were recorded on a Shimadzu GC MS-QP 1000 EI mass spectrometer at 70 eV. Compound **1** was prepared in accordance with the previous report.[48]

3-Amino-4-cyano-5-(methylthio)thiophene-2-carboxamide (2)[49,56]

To a solution of ethanolic sodium ethoxide [prepared by dissolving sodium metal (5 mmol) in absolute ethanol (30 mL)], compound **1** (5 mmol) was added and the solution was then heated under reflux for 10 min. The methyl iodide (6 mmol) was added and refluxing was continued for additional 2 h. The reaction mixture was then cooled, poured onto cold water, neutralized with dilute hydrochloric acid, whereby the product that separated out was filtered off, dried and recrystallized from ethanol–dioxane mixture as a pale yellow solid, yield 79% (0.84 g), mp 225–227 °C; IR (ν/cm^{-1}): 3422, 3401, 3320, 3265 (2 × NH_2), 2208 (CN), 1660 (CO); ^{1}H NMR (δ ppm): 2.71 (s, 3H, SMe), 6.70 (br s, 2H, NH_2, D_2O-exchangeable), 7.30 (br s, 2H, $CONH_2$, D_2O-exchangeable); Anal. Calcd. for $C_7H_7N_3OS_2$ (213.280): C, 39.42; H, 3.31; N, 19.70; S, 30.07. Found: C, 39.24; H, 3.19; N, 19.46; S, 29.85.

3,4-Diamino-5-cyano-6-oxo-6,7-dihydrothieno[2,3-*b*]pyridine-2-carboxamide (3)

A mixture of **2** (3 mmol) and cyanoacetamide (3 mmol) in pyridine (20 mL) was heated under reflux for 4 h, poured onto cold water and neutralized with dilute acetic acid. The solid product obtained was filtered off, dried and recrystallized from ethanol as a golden yellow solid, yield 67% (0.50 g), mp 281–283 °C; IR (ν/cm^{-1}): 3450–3162 (3 × NH_2, NH), 2221 (CN), 1670, 1660 (2 × CO); ^{1}H NMR (δ ppm): 4.75 (s, 2H, NH_2, D_2O-exchangeable), 6.07 (br s, 2H, NH_2, D_2O-exchangeable), 7.04 (br s, 2H, $CONH_2$, D_2O-exchangeable), 12.19 (s, 1H, NH, D_2O-exchangeable); ^{13}C NMR (δ ppm): 91.2 (C-5), 116.3 (CN), 120.1, 124.7, 151.6, 159.8, 162.4, 165.2, 167.5;

MS: *m/z* (%) 249 (M^+, 27); Anal. Calcd. for $C_9H_7N_5O_2S$ (249.249): C, 43.37; H, 2.83; N, 28.10; S, 12.86. Found: C, 43.14; H, 2.71; N, 27.91; S, 12.70.

General Procedure for the Synthesis of 4a and 4b

A mixture of equimolar amounts (5 mmol) of 5-(methylthio)thiophene derivative **2** and either *p*-chloroaniline or *p*-toluidine was fused in an oil bath, the temperature was raised gradually and kept at 170–180 °C for 3 h. After cooling, the reaction products were triturated with ether and the solid products that separated out were collected by filtration and recrystallized from the proper solvents.

3-Amino-5-(*p*-chlorophenylamino)-4-cyanothiophene-2-carboxamide (4a)

This compoud was obtained as a light brown solid (acetone), yield 37% (0.54 g), mp 151–152 °C; IR (ν/cm^{-1}): 3454–3282 (2 × NH_2, NH), 3057 (CH arom), 2207 (CN), 1662 (CO); ^{1}H NMR (δ ppm): 6.81 (br s, 2H, NH_2, D_2O-exchangeable), 7.18 (br s, 2H, $CONH_2$, D_2O-exchangeable), 7.41–7.80 (m, 4H, Ar-H), 8.95 (s, 1H, NH, D_2O-exchangeable); ^{13}C NMR (δ ppm): 79.4 (C-4), 115.7 (CN), 120.5, 121.8, 128.0, 129.5, 137.6, 151.0, 156.2, 164.3; Anal. Calcd. for $C_{12}H_9ClN_4OS$ (292.744): C, 49.23; H, 3.10; Cl, 12.11; N, 19.14; S, 10.95. Found: C, 48.98; H, 3.01; Cl, 11.89; N, 19.03; S, 10.80.

3-Amino-4-cyano-5-(*p*-tolylamino)thiophene-2-carboxamide (4b)

This compoud was obtained as a yellow solid (ethanol–dioxane), yield 46% (0.63 g), mp 126–128 °C; IR (ν/cm^{-1}): 3449–3273 (2 × NH_2, NH), 3060 (CH arom), 2207 (CN), 1661 (CO); ^{1}H NMR (δ ppm): 2.21 (s, 3H, Me-Ar), 6.58 (br s, 2H, NH_2, D_2O-exchangeable), 6.97 (br s, 2H, $CONH_2$, D_2O-exchangeable), 7.27–7.43 (m, 4H, Ar-H), 8.78 (s, 1H, NH, D_2O-exchangeable); ^{13}C NMR (δ ppm): 21.4 (Me-Ar), 80.3 (C-4), 116.9 (CN), 119.6, 121.0, 130.1, 130.8, 136.2, 150.8, 156.5, 164.7; MS: *m/z* (%) 272 (M^+, 16); Anal. Calcd. for $C_{13}H_{12}N_4OS$ (272.326): C, 57.34; H, 4.44; N, 20.57; S, 11.77. Found: C, 57.07; H, 4.32; N, 20.36; S, 11.59.

General Procedure for the Synthesis of 5a and 5b

A mixture of either **4a** or **4b** (5 mmol) and formic acid (85%, 20 mL), was refluxed for 10 h. The precipitates that formed on cooling to room temperature, were collected by filtration and recrystallized from the appropriate solvents.

6-(*p*-Chlorophenylamino)-4-oxo-3,4-dihydrothieno[3,2-*d*]pyrimidine-7-carboxamide (5a)

This compoud was obtained as an orange solid (dimethylformamide), yield 32% (0.51 g), mp 199–201 °C; IR (ν/cm^{-1}): 3430–3209 (NH_2, 2NH), 3055 (CH arom), 1674, 1659 (2CO), 1619 (C=N); ^{1}H NMR (δ ppm): 7.30–7.59 (m, 4H, Ar-H), 7.73 (s, 2H, $CONH_2$, D_2O-exchangeable), 8.05 (s, 1H, CH pyrimidine), 8.62 (s, 1H, NH, D_2O-exchangeable), 12.11 (s, 1H, NH, D_2O-exchangeable); MS: *m/z* (%) 322 (M^++2, 13), 320 (M^+, 30); Anal. Calcd. for $C_{13}H_9ClN_4O_2S$ (320.754): C, 48.68; H, 2.83; Cl, 11.05; N, 17.47; S, 10.00. Found: C, 48.43; H, 2.59; Cl, 10.88; N, 17.33; S, 9.87.

4-Oxo-6-(*p*-tolylamino)-3,4-dihydrothieno[3,2-*d*]pyrimidine-7-carboxamide (5b)

This compoud was obtained as a pale brown solid (acetic acid), yield 55% (0.83 g), mp 144–145 °C; IR (ν/cm^{-1}): 3427–3216 (NH_2, 2 × NH), 3051 (CH arom), 1673, 1661 (2 × CO), 1619 (C=N); ^{1}H NMR (δ ppm): 2.30 (s, 3H, Me-Ar), 7.18–7.35 (m, 4H, Ar-H), 7.54 (s, 2H, $CONH_2$, D_2O-exchangeable), 8.14 (s, 1H, CH pyrimidine), 8.86 (s, 1H, NH, D_2O-exchangeable), 12.53 (s, 1H, NH, D_2O-exchangeable); ^{13}C NMR (δ ppm): 22.5 (Me-Ar), 108.7 (C-7), 112.6, 119.9, 130.2, 131.6, 137.1, 144.5, 149.3, 156.7, 161.8 (CO pyrimidine), 167.4 (CO amide); MS: *m/z* (%) 300 (M^+, 24); Anal. Calcd. for $C_{14}H_{12}N_4O_2S$ (300.336): C, 55.99; H, 4.03; N, 18.65; S, 10.68. Found: C, 55.75; H, 3.92; N, 18.46; S, 10.41.

General Procedure for the Synthesis of 6a and 6b

To a solution of *p*-toluidinothiophene **4b** (5 mmol) and anhydrous potassium carbonate (10 mmol), in dimethylformamide (20 mL), either ethyl chloroacetate or chloroacetamide (5.5 mmol) was added. The reaction mixture was heated on an oil bath at 125–130C with stirring for 2 h. After cooling, triethylamine (0.5 mL) was added and the mixture obtained was stirred at room temperature for 3 h. The reaction mixture was then diluted with cold water and allowed to stand overnight. The resulting precipitate, in each case, was filtered off, dried and recrystallized from the proper solvents.

Ethyl 3,4-Diamino-2-carboxamido-6-(*p*-tolyl)-6*H*-thieno[2,3-*b*]pyrrole-5-carboxylate (6a)

This compoud was obtained as a pale yellow solid (methanol), yield 46% (0.82 g), mp 107–108 °C; IR (ν/cm^{-1}): 3446–3239 (3 × NH_2), 3048 (CH arom), 1701, 1658 (2 × CO); ^{1}H NMR (δ ppm): 1.36 (t, 3H, J = 7.1 Hz, Me ester), 2.23 (s, 3H, Me-Ar), 4.37 (q, 2H, J = 7.1 Hz, CH_2 ester), 6.12 (s, 2H, NH_2, D_2O-exchangeable), 6.91 (br s, 2H, NH_2, D_2O-exchangeable), 7.14 (br s, 2H, $CONH_2$, D_2O-exchangeable), 7.28–7.50 (m, 4H, Ar-H); MS: *m/z* (%) 358 (M^+, 20); Anal. Calcd. for $C_{17}H_{18}N_4O_3S$ (358.415): C, 56.97; H, 5.06; N, 15.63; S, 8.95. Found: C, 56.78; H, 4.94; N, 15.40; S, 8.81.

3,4-Diamino-6-(*p*-tolyl)-6*H*-thieno[2,3-*b*]pyrrole-2,5-dicarboxamide (6b)

This compoud was obtained as a colorless solid (dioxane), yield 51% (0.84 g), mp 165–167 °C; IR (ν/cm^{-1}): 3437–3255 (4 × NH_2), 3052 (CH arom), 1663,

1658 (2 × CO); ^{1}H NMR (δ ppm): 2.40 (s, 3H, Me-Ar), 5.79 (s, 2H, NH_2, D_2O-exchangeable), 6.77 (br s, 2H, NH_2, D_2O-exchangeable), 7.21 (br s, 2H, $CONH_2$, D_2O-exchangeable), 7.32–7.50 (m, 4H, Ar-H), 7.96 (s, 2H, $CONH_2$, D_2O-exchangeable); MS: *m/z* (%) 329 (M^+, 14); Anal. Calcd. for $C_{15}H_{15}N_5O_2S$ (329.377): C, 54.70; H, 4.59; N, 21.26; S, 9.74. Found: C, 54.42; H, 4.61; N, 21.04; S, 9.52.

8-Phenyl-7-thioxo-6-(*p*-tolyl)-7,8-dihydrothieno[2,3-*d*:4,5-*d'*]dipyrimidine-4,9(3*H*,6*H*)-dione (8)

To a solution of *p*-toluidinothiophene **5b** (2 mmol) in ethanolic sodium ethoxide [prepared by dissolving sodium metal (2 mmol) in absolute ethanol (25 mL)], phenyl isothiocyanate (2 mmol) was added dropwise. The mixture was refluxed with stirring for 10 h and then left to cool to room temperature overnight under stirring. The reaction mixture was then poured onto ice–water and neutralized with dilute hydrochloric acid, whereby the resulting solid product was filtered off, dried and recrystallized from dimethylformamide as a yellow solid, yield 64% (0.54 g), mp 212 °C; IR (ν/cm^{-1}): 3150 (NH), 3065 (CH arom), 1691, 1672 (2 × CO), 1616 (C=N), 1290 (C=S); ^{1}H NMR (δ ppm): 2.18 (s, 3H, Me-Ar), 6.65–7.39 (m, 9H, Ar-H), 8.34 (s, 1H, CH pyrimidine), 12.15 (s, 1H, NH, D_2O-exchangeable); ^{13}C NMR (δ ppm): 20.8 (Me-Ar), 112.3, 118.2, 127.4, 128.1, 128.7, 129.6, 132.5, 135.0, 136.4, 137.3, 144.0, 149.1, 155.3, 161.2 (CO ring), 162.4 (CO ring), 174.0 (CS ring); MS: *m/z* (%) 418 (M^+, 23); Anal. Calcd. for $C_{21}H_{14}N_4O_2S_2$ (418.491): C, 60.27; H, 3.37; N, 13.39; S, 15.32. Found: C, 60.04; H, 3.21; N, 13.18; S, 15.16.

7-Thioxo-6-(*p*-tolyl)-7,8-dihydrothieno[2,3-*d*:4,5-*d'*]dipyrimidine-4,9(3*H*,6*H*)-dione (10)

A mixture of compound **5b** (5 mmol) and carbon disulfide (2 mL, 33 mmol) in dry pyridine (20 mL) was heated under reflux until the evolution of hydrogen sulfide ceased (8 h). The reaction mixture was allowed to cool, poured onto iced water and acidified with dilute hydrochloric acid. The precipitated crystals of the product **10** were filtered off, dried and recrystallized from dimethylformamide as a dark brown solid, yield 39% (0.67 g), mp 240–242 °C; IR (ν/cm^{-1}): 3240–3186 (2 × NH), 3060 (CH arom), 1676, 1672 (2 × CO), 1620 (C=N), 1252 (C=S); ^{1}H NMR (δ ppm): 2.32 (s, 3H, Me-Ar), 6.70–7.02 (m, 4H, Ar-H), 8.21 (s, 1H, CH pyrimidine), 11.97 (s, 1H, NH, D_2O-exchangeable), 12.34 (s, 1H, NH, D_2O-exchangeable); ^{13}C NMR (δ ppm): 21.6 (Me-Ar), 113.0, 116.5, 129.1, 134.8, 136.7, 137.6, 144.8, 149.7, 155.0, 159.2 (CO ring), 160.8 (CO ring), 174.7 (CS ring); MS: *m/z* (%) 342 (M^+, 15); Anal. Calcd. for $C_{15}H_{10}N_4O_2S_2$ (342.396): C, 52.62; H, 2.94; N, 16.36; S, 18.73. Found: C, 52.36; H, 2.79; N, 16.17; S, 18.47.

7-(Methylthio)-6-(*p*-tolyl)thieno[2,3-*d*:4,5-*d'*]dipyrimidine-4,9(3*H*,6*H*)-dione (11)

Method A. To a solution of ethanolic sodium ethoxide [prepared by dissolving sodium metal (2 mmol) in absolute ethanol (20 mL)], compound **10** (2 mmol) was added and the solution was then heated under reflux for 10 min. The methyl iodide (3 mmol) was added and refluxing was continued for additional 2 h. The reaction mixture was then cooled, poured onto cold water, neutralized with dilute hydrochloric acid, whereby the product that separated out was filtered off, dried and recrystallized from dilute dioxane to give the tricyclic derivative **11** as an orange solid, yield 72% (0.51 g), mp 263–266 °C; IR (ν/cm^{-1}): 3166 (NH), 3056 (CH arom), 1689, 1671 (2 × CO), 1620 (C=N); ^{1}H NMR (δ ppm): 2.25 (s, 3H, Me-Ar), 2.68 (s, 3H, SMe), 6.67–7.02 (m, 4H, Ar-H), 8.21 (s, 1H, CH pyrimidine), 12.04 (s, 1H, NH, D_2O-exchangeable); ^{13}C NMR (δ ppm): 12.7 (SMe), 21.0 (Me-Ar), 112.6, 118.5, 127.1, 129.5, 131.3, 141.6, 144.2, 148.9, 156.8, 161.6 (CO ring), 163.0 (CO ring), 165.4 (C-7); Anal. Calcd. for $C_{16}H_{12}N_4O_2S_2$ (356.422): C, 53.92; H, 3.39; N, 15.72; S, 17.99. Found: C, 53.68; H, 3.17; N, 15.45; S, 18.01.

Method B. To a stirred solution of **5b** (2 mmol) and about 20% potassium hydroxide solution (potassium hydroxide, 0.34 g, 6 mmol, water 1.5 mL) in 10 mL dimethylformamide, carbon disulfide (3 mmol) was added in several portions during 10 min. The reaction mixture was heated at reflux for 2 h then methyl iodide (6 mmol) was slowly added over a period of 10 min and refluxing was continued for an extra hour with stirring. After cooling, the reaction mixture was left aside for 1 h under stirring at room temperature. The mixture was then poured onto 100 mL iced water, neutralized with dilute hydrochloric acid. The precipitate was collected by filtration and washed several times with water. This crude product was recrystallized from dilute dioxane to produce, upon air drying, an orange product (0.45 g; 63%) identical in all aspects (mp, mixed mp, and IR data) to that described in method A.

***N*-(7-Cyano-4-oxo-3,4-dihydrothieno[3,2-*d*]pyrimidin-6-yl)-*N*-(*p*-tolyl)acetamide (14)**

A sample of compound **5b** (2 mmol) in acetic anhydride (10 mL) was boiled under reflux for 8 h and then allowed to cool. Under stirring, the reaction mixture was poured over an ice–water mixture (100 mL). The precipitate that formed was collected by filtration and washed with ethanol and water. Recrystallization from methanol gave the pure product as yellowish white crystals, yield 79% (0.51 g), mp 170–171 °C; IR (ν/cm^{-1}): 3158 (NH), 3047 (CH arom), 2216 (CN), 1677, 1670 (2 × CO), 1614 (C=N); ^{1}H NMR (δ ppm): 2.18 (s, 3H, Me), 2.45 (s, 3H, Me), 7.33–7.75 (m, 4H, Ar-H), 8.22 (s, 1H, CH pyrimidine), 12.64 (s, 1H, NH, D_2O-exchangeable); ^{13}C NMR (δ ppm): 21.5 (Me-Ar), 23.1 (Me acetyl), 84.3 (C-7), 111.9, 116.4 (CN), 129.0, 132.7, 136.4, 136.9, 144.8, 149.2, 150.4, 161.6 (CO ring), 168.1 (CO acetyl); MS: *m/z* (%) 324 (M^+, 22); Anal. Calcd. for $C_{16}H_{12}N_4O_2S$ (324.357): C,

59.25; H, 3.73; N, 17.27; S, 9.89. Found: C, 58.99; H, 3.61; N, 17.04, S, 9.75.

7-Methyl-6-(*p*-tolyl)thieno[2,3-*d*:4,5-*d'*]dipyrimidine-4,9(3*H*,6*H*)-dione (13)

A mixture of **5b** (2.5 mmol) and acetylacetone (2.5 mmol), in ethanol (30 mL) containing a few drops of concentrated hydrochloric acid, was refluxed for 2 h. The reaction mixture after cooling was triturated with ether. The resulting solid was collected by suction and purified by recrystallization from dimethylformamide–ethanol as a brown solid, yield 58% (0.47 g), mp 277–279 °C; IR (ν/cm^{-1}): 3155 (NH), 3041 (CH arom), 1690, 1674 (2 × CO), 1617 (C=N); ^{1}H NMR (δ ppm): 2.30 (s, 3H, Me), 2.42 (s, 3H, Me), 6.70–7.12 (m, 4H, Ar-H), 8.27 (s, 1H, CH pyrimidine), 12.55 (s, 1H, NH, D_2O-exchangeable); ^{13}C NMR (δ ppm): 21.9 (Me-Ar), 23.0 (Me pyrimidine), 112.5, 118.2, 127.3, 129.4, 130.8, 141.7, 144.8, 149.6, 153.5, 156.7, 161.1 (CO), 162.9 (CO); Anal. Calcd. for $C_{16}H_{12}N_4O_2S$ (324.357): C, 59.25; H, 3.73; N, 17.27; S, 9.89. Found: C, 59.06; H, 3.49; N, 17.09; S, 9.78.

Ethyl 2-(4,9-Dioxo-6-(*p*-tolyl)-3,4,6,9-tetrahydrothieno [2,3-*d*:4,5-*d'*]dipyrimidin-7-yl)acetate (15a)

A suspension of compound **5b** (2 mmol) in diethyl malonate (10 mL) was gently heated under reflux for 10 h. The reaction mixture was triturated with ethanol (15 mL) and then allowed to cool. The formed precipitate was collected by filtration and purified by recrystallization from ethanol–water as a golden yellow solid, yield 78% (0.62 g), mp 120–121 °C; IR (ν/cm^{-1}): 3160 (NH), 3038 (CH arom), 1727, 1692, 1673 (3 × CO), 1622 (C=N); ^{1}H NMR (δ ppm): 1.21 (t, 3H, *J* = 7.0 Hz, Me ester), 2.31 (s, 3H, Me-Ar), 4.25 (q, 2H, *J* = 7.0 Hz, CH_2 ester), 5.01 (s, 2H, CH_2CO), 6.73–7.11 (m, 4H, Ar-H), 8.20 (s, 1H, CH pyrimidine), 12.05 (s, 1H, NH, D_2O-exchangeable); MS: *m/z* (%) 396 (M^+, 26); Anal. Calcd. for $C_{19}H_{16}N_4O_4S$ (396.420): C, 57.57; H, 4.07; N, 14.13; S, 8.09. Found: C, 57.36; H, 3.91; N, 13.94; S, 7.96.

2-(4,9-Dioxo-6-(*p*-tolyl)-3,4,6,9-tetrahydrothieno[2,3-*d*:4,5-*d'*]dipyrimidine-7-yl)acetonitrile (15b)

To a solution of compound **5b** (2 mmol) in ethanolic sodium ethoxide [prepared by dissolving sodium metal (2 mmol) in absolute ethanol (25 mL)], ethyl cyanoactate (2 mmol) was added dropwise. The reaction mixture was refluxed with stirring for 8 h and then left to cool to room temperature under stirring. The mixture was then acidified with diluted hydrochloric acid, whereby the separated solid was isolated by filtration and recrystallized from dioxane as a brown solid, yield 74% (0.52 g), mp 251–254 °C; IR (ν/cm^{-1}): 3151 (NH), 3044 (CH arom), 2230 (CN), 1691, 1673 (2 × CO), 1618 (C=N); ^{1}H NMR (δ ppm): 2.27 (s, 3H, Me-Ar), 4.52 (s, 2H, CH_2CN), 6.73–7.06 (m, 4H, Ar-H), 8.35 (s, 1H, CH pyrimidine), 12.44 (s, 1H, NH, D_2O-exchangeable); ^{13}C NMR (δ ppm): 20.3 (CH_2), 22.1 (Me-Ar), 112.5, 115.7 (CN), 118.8, 126.9, 130.4, 132.0, 142.8, 144.3, 149.0, 153.1, 156.2, 160.9 (CO), 162.5 (CO); Anal. Calcd. for $C_{17}H_{11}N_5O_2S$ (349.367): C, 58.44; H, 3.17; N, 20.05; S, 9.18. Found: C, 58.27; H, 3.01; N, 19.81; S, 8.94.

2-(4,9-Dioxo-6-(*p*-tolyl)-3,4,6,9-tetrahydrothieno[2,3-*d*:4,5-*d'*]dipyrimidin-7-yl)acetic acid (15c)

A mixture of compound **5b** (3 mmol) and potassium hydroxide (7 mmol) in absolute ethanol (20 mL) was refluxed for 1 h. After cooling, the precipitate was filtered off, dissolved in water and then acetic acid was added until precipitation was complete. The material which separated upon cooling was isolated by filtration as a pale yellow solid, yield 53% (0.59 g), mp 230–231 °C; IR (ν/cm^{-1}): 3441–3173 (OH, NH), 3036 (CH arom), 1694, 1670, 1645 (3 × CO), 1618 (C=N); ^{1}H NMR (δ ppm): 2.27 (s, 3H, Me-Ar), 4.81 (s, 2H, CH_2CO), 6.61–7.03 (m, 4H, Ar-H), 8.23 (s, 1H, CH pyrimidine), 12.33 (s, 1H, NH, D_2O-exchangeable), 12.70 (br s, 1H, OH, D_2O-exchangeable); ^{13}C NMR (δ ppm): 20.7 (Me-Ar), 42.6 (CH_2), 113.4, 118.3, 128.1, 130.4, 132.0, 141.2, 145.0, 149.4, 152.7, 156.8, 161.5 (CO), 163.1 (CO), 173.2 (COOH); Anal. Calcd. for $C_{17}H_{12}N_4O_4S$ (368.367): C, 55.43; H, 3.28; N, 15.21; S, 8.70. Found: C, 55.18; H, 3.09; N, 14.97; S, 8.56.

6-(*p*-Tolyl)pyrimido[4',5':4,5]thieno[2,3-*b*]pyrimido[4,5-*d*]pyrrol-4,7(3*H*,8*H*)-dione (16)

Method A. A mixture of compound **6b** (2 mmol) and triethyl orthoformate (15 mL) was heated at reflux in acetic anhydride (10 mL) for 12 h, cooled to room temperature and then diluted with cool water. The resulting solid product was filtered off, dried and recrystallized from dimethylformamide to give **16** as a light brown solid, yield 76% (0.53 g), mp 271–272 °C; IR (ν/cm^{-1}): 3295–3112 (2 × NH), 3046 (CH arom), 1674, 1669 (2 × CO), 1623 (C=N); ^{1}H NMR (δ ppm): 2.39 (s, 3H, Me-Ar), 7.12–7.39 (m, 4H, Ar-H), 8.06 (s, 1H), 8.21 (s, 1H) (2 × CH pyrimidine), 12.20 (s, 1H), 12.44 (s, 1H) (2 × NH, D_2O-exchangeable); MS: *m/z* (%) 349 (M^+, 17); Anal. Calcd. for $C_{17}H_{11}N_5O_2S$ (349.367): C, 58.44; H, 3.17; N, 20.05; S, 9.18. Found: C, 58.31; H, 3.02; N, 19.86; S, 8.95.

Method B. Compound **6b** (1 mmol) in dry dimethylformamide (10 mL) was treated with dimethylformamide dimethylacetal (2.5 mmol) portionwise. The reaction mixture was stirred under reflux for 6 h and then left to cool. Stirring was continued at room temperature for additional 12 h. The solvent was removed by evaporation under *vacuo* to dryness. The residual semisolid was triturated with petroleum ether, whereby the solid product formed was filtered off, dried and recrystallized from dimethylformamide to give a tricyclic product (0.29 g; 83%) that was found to be identical in all aspects (mp, mixed mp, and IR data) with the product **16** prepared by method A.

Method C. A mixture of **6b** (2 mmol) and formic acid (20 mL), was refluxed for 10 h. The precipitate that

formed on cooling to room temperature, was collected by filtration and recrystallized from dimethylformamide to give a solid product (0.42 g; 60%). Again, this product was identified as **16**.

Method D. A solution of **6a** (2 mmol) and formamide (10 mL), was refluxed for 6 h. The solvent was removed under vacuum. The solid obtained was collected by filtration, washed with water, dried and recrystallized from dimethylformamide to give a solid product (0.44 g; 63%). The material proved to be **16**.

General Procedure for the Synthesis of 17a and 17b

Method A for compounds **17a**,**b**. To a solution of either **6a** or **6b** (2 mmol) in benzene (30 mL), trichloroacetonitrile (4 mmol) was added dropwise under stirring. The reaction mixture was then heated at reflux for 3 h. After cooling, the mixture was poured over cold water (50 mL), whereby the resulting precipitate, in each case, was filtered off, dried and recrystallized from the appropriate solvents.

2-Amino-6-(*p*-tolyl)-9-trichloromethylpyrimido[4',5':4,5]thieno[2,3-*b*]pyrimido[4,5-*d*]pyrrol-4,7(3*H*,8*H*)-dione (17a)

This compoud was obtained as a drak brown solid (acetic acid), yield 72% (0.69 g), mp > 300 °C; IR (ν/cm^{-1}): 3350–3107 (NH_2, 2 × NH), 3050 (CH arom), 1674, 1670 (2 × CO), 1624 (C=N); ^{1}H NMR (δ ppm): 2.17 (s, 3H, Me-Ar), 6.48 (s, 2H, NH_2, D_2O-exchangeable), 7.21–7.40 (m, 4H, Ar-H), 10.51 (s, 1H), 12.45 (s, 1H) (2 × NH, D_2O-exchangeable); Anal. Calcd. for $C_{18}H_{11}Cl_3N_6O_2S$ (481.743): C, 44.88; H, 2.30; Cl, 22.08; N, 17.45; S, 6.66. Found: C, 44.75; H, 2.11; Cl, 21.86; N, 17.18; S, 6.45.

2,9-Diamino-6-(*p*-tolyl)pyrimido[4',5':4,5]thieno[2,3-*b*]pyrimido[4,5-*d*]pyrrol-4,7(3*H*,8*H*)-dione (17b)

This compoud was obtained as an orange solid (benzene), yield 66% (0.50 g), mp 285 °C; IR (ν/cm^{-1}): 3356–3100 (2 × NH_2, 2NH), 3050 (CH arom), 1673, 1670 (2 × CO), 1621 (C=N); ^{1}H NMR (δ ppm): 2.25 (s, 3H, Me-Ar), 6.51 (s, 2H), 6.68 (s, 2H) (2 × NH_2, D_2O-exchangeable), 7.28–7.52 (m, 4H, Ar-H), 10.14 (s, 1H), 10.32 (s, 1H) (2 × NH, D_2O-exchangeable); MS: *m/z* (%) 379 (M^+, 21); Anal. Calcd. for $C_{17}H_{13}N_7O_2S$ (379.396): C, 53.82; H, 3.45; N, 25.84; S, 8.45. Found: C, 53.56; H, 3.32; N, 25.58; S, 8.32.

Method B for compound **17b**. A mixture of compound **17a** (3 mmol) and ammonium hydroxide solution 30–40% (6 mL) was stirred under reflux in methanol (15 mL) for 2 h. The reaction mixture was allowed to cool to room temperature, poured over cold water (50 mL) and neutralized by the addition of acetic acid, whereupon the solid precipitated was filtered off, dried and recrystallized from benzene to give a solid product (0.36 g; 32%), identical (mp, mixed mp, and IR data) to that obtained by method A.

2,4,7,9-Tetraoxo-6-(*p*-tolyl)-1,2,3,4,7,8,9,10-octahydropyrimido[4',5':4,5]thieno[2,3-*b*]pyrimido[4,5-*d*]pyrrole (18a)

Method A. A finely ground mixture of 0.4 g (1.1 mmol) of **6a** and 1.6 g of urea were heated at 180 °C for 20 min. At this temperature, the mixture melted and resolidified. The solid mass was dissolved in warm 5% sodium hydroxide, filtered and the cooled basic filtrate was then carefully acidified with acetic acid. The solution was allowed to stand approximately 10 min and was then filtered and air dried. Further purification was accomplished by reprecipitation from a hot basic solution with acetic acid. A small amount was recrystallized from a large volume of water and dried to yield the tetracyclic product **18a** as a yellowish white solid, 0.37 g (89%), mp > 300 °C; IR (ν/cm^{-1}): 3170–3068 (4 × NH), 3042 (CH arom), 1705–1680 (4 × CO); ^{1}H NMR (δ ppm): 2.25 (s, 3H, Me-Ar), 7.21–7.47 (m, 4H, Ar-H), 10.82 (s, 1H), 10.91 (s, 1H) (2 × NH, D_2O-exchangeable), 11.28 (s, 1H), 11.50 (s, 1H) (2 × NH, D_2O-exchangeable); MS: *m/z* (%) 381 (M^+, 28); Anal. Calcd. for $C_{17}H_{11}N_5O_4S$ (381.365): C, 53.54; H, 2.91; N, 18.36; S, 8.41. Found: C, 53.29; H, 2.75; N, 18.14; S, 8.27.

Method B. The same experimental procedure described above in method A was followed except using 2,5-dicarboxamide derivative **6b** (0.4 g; 1.2 mmol) instead of **6a**. The product obtained (0.35 g; 77%) was found to be identical with an authentic sample prepared according to method A.

Method C. To a mixture of 15 mL of pyridine and 0.5 g (1.5 mmol) of **6b** was carefully added 2 mL of ethyl chloroformate and the mixture was refluxed for 48 h. After cooling, the solution was concentrated and then water was added to produce a precipitate which was filtered off, and purified by repeated dissolution in aqueous base and precipitation by dilute acid to give a solid product (0.30 g; 53%) mp > 300 C, mp and mixed mp with the product from either method A or B gave no depression.

4,7-Dioxo-2,9-dithioxo-6-(*p*-tolyl)-1,2,3,4,7,8,9,10-octahydropyrimido[4',5':4,5]thieno[2,3-*b*]pyrimido[4,5-*d*]pyrrole (18b)

Either **6a** or **6b** (0.4 g) was fused with 1.6 g of thiourea in the same manner as described in method A for the preparation of **18a**. The crude product, in each case, was further purified by reprecipitation of a small sample from hot dilute sodium hydroxide with acetic acid to give **18b** as brown crystals, yield 69–78%, mp 290–292 °C; IR (ν/cm^{-1}): 3440–3193 (4 × NH), 3048 (CH arom), 1660, 1656 (2 × CO), 1305, 1301 (2 × C=S); ^{1}H NMR (δ ppm): 2.43 (s, 3H, Me-Ar), 7.21–7.55 (m, 4H, Ar-H), 12.37 (s, 1H), 12.50 (s, 1H) (2 × NH, D_2O-exchangeable), 13.28 (s, 1H), 13.46 (s, 1H) (2 × NH, D_2O-exchangeable); MS: *m/z* (%) 413 (M^+, 18); Anal. Calcd. for $C_{17}H_{11}N_5O_2S_3$ (413.497): C, 49.38; H, 2.68; N, 16.94; S, 23.26. Found: C, 49.13; H, 2.47; N, 16.80; S, 23.02.

2,9-Diphenyl-6-(*p*-tolyl)pyrimido[4',5':4,5]thieno[2,3-*b*]pyrimido[4,5-*d*]pyrrol-4,7(3*H*,8*H*)-dione (19)

Method A. A mixture of **6b** (2 mmol) and benzoyl chloride (10 mL) was heated at reflux temperature for 4 h. The excess of benzoyl chloride was extracted with benzene and the residue was recrystallized from dimethylformamide–water to give the tetracyclic product **19** as a dark brown solid, yield 57% (0.57 g), mp > 300 °C; IR (ν/cm^{-1}): 3291–3108 (2 × NH), 3067 (CH arom), 1676, 1673 (2 × CO), 1619 (C=N); ^{1}H NMR (δ ppm): 2.30 (s, 3H, Me-Ar), 7.18–7.80 (m, 14H, Ar-H), 11.38 (s, 1H), 11.52 (s, 1H) (2 × NH, D_2O-exchangeable); MS: *m/z* (%) 501 (M^+, 23); Anal. Calcd. for $C_{29}H_{19}N_5O_2S$ (501.558): C, 69.45; H, 3.82; N, 13.96; S, 6.39. Found: C, 69.21; H, 3.67; N, 13.81, S; 6.22.

Method B. Compound **6b** (3 mmol) was suspended in ethanol (20 mL). Benzaldehyde (6 mmol) and concentrated hydrochloric acid (0.5 mL) were added and the reaction mixture was heated at reflux for 20 h and then left aside to cool overnight under stirring. The reaction mixture was then concentrated and the solid product was filtered off, washed with water and recrystallized from dimethylformamide–water to produce a solid product (0.63 g; 42%) mp > 300 °C, mp and mixed mp with the product from method A gave no depression.

6-(*p*-Tolyl)triazino[4',5':4,5]thieno[2,3-*b*]triazino[4,5-*d*]pyrrol-4,7(3*H*,8*H*)-dione (20)

Compound **6b** (3 mmol) was dissolved in glacial acetic acid (10 mL) containing concentrated hydrochloric acid (1 mL), a small amount of insoluble material was filtered off, then the liquid was cooled in ice bath at 0–5 °C. The mixture was stirred at this temperature and treated gradually with a cold saturated solution of sodium nitrite (30 mmol in 20 mL of water) over a period of 30 min. The mixture was kept in ice bath at 0–5 °C with continuous stirring for further 2 h, then it was left to stand overnight at room temperature and diluted with water, whereupon precipitation took place. The solid thus formed was isolated by filtration, washed abundantly with cold water, recrystallized from aqueous acetone and air dried to give the fused triazine derivative **20** as a pale brown solid, yield 53% (0.56 g), mp 140–141 C; IR (ν/cm^{-1}): 3410–3328 (2 × NH), 3056 (CH arom), 1685, 1680 (2 × CO); ^{1}H NMR (δ ppm): 2.26 (s, 3H, Me-Ar), 7.17–7.45 (m, 4H, Ar-H), 14.59 (br s, 1H), 14.81 (br s, 1H) (2 × NH, D_2O-exchangeable); MS: *m/z* (%) 351 (M^+, 12); Anal. Calcd. for $C_{15}H_9N_7O_2S$ (351.343): C, 51.28; H, 2.58; N, 27.91; S, 9.13. Found: C, 51.07; H, 2.41; N, 27.64; S, 8.92.

6. Computational Details

Geometry optimization of the synthesized structures was carried out using the semi-empirical Austin Model (AM1) Hamiltonian at a restricted Hartree–Fock (RHF) self-consistent field level using the GAMESS package.[54] A gradient tolerance of 10^{-4} Hartree Å^{-1} was used throughout. The rotational energy profiles were obtained by constraining the –N11–C12– dihedral (Table 1) to discreet values while the rest of the degrees of freedom were subjected to full geometry optimization.

The electrostatic component of the solvation free energy (ΔA_{solv}) was calculated by solving the Poisson–Boltzmann (PB) equation[55] for the geometry optimized structures using the APBS package.[57] The ΔA_{solv} values were obtained from the difference between the electrostatic potential computed in bulk solvent (salt concentration = 0.15 M, relative dielectric permittivity = 80) and the electrostatic potential calculated in vacuum (salt concentration = 0.0 M, relative dielectric permittivity = 1.0) at 310 K. In order to increase the accuracy of the calculations, the focusing technique[58] was used where three focussing stages were carried out. A final grid spacing of 0.3 Å was used and the relative dielectric permittivity within the structures was set to 1. The solute-solvent boundary was constructed from the solvent accessible surface using a solvent probe radius of 1.4 Å.

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Povzetek

Iz ustreznih tiofen-2-karboksamidov **4a**,**b** smo pripravili nove derivate tieno[3,2-*d*]pirimidina in tieno[2,3-*b*]pirola **5a**,**b** and **6a**,**b**. Iz spojin **5b**, **6a** in **6b** smo s pomočjo ciklizacije z vrsto komercialno dostopnih reaktantov sintetizirali dve novi seriji kondenziranih pirimidinov iz treh (**8**–**15**) oz. štirih (**16**–**19**) obročev. Geometrijska optimizacija izbranih struktur s pomočjo AM1 semiempiričnih metod je razkrila, da imajo tetraciklični pirimidinski derivati manjši ionizacijski potencial in nižjo stopnjo konformacijske svobode kot pa triciklični analogi. Zanimivo je, da je računska študija solvatacijske proste energije konformera z najnižjo energijo pri fizioloških pogojih pokazala, da je celotna serija zelo dobro topna pod takimi pogoji. Trend topnosti, kot se kaže v relativnih velikostih solvatacijskih prostih energij, nakazuje na večji prispevek višjih momentov razporeditve naboja pri modulaciji interakcije teh struktur z bioloških okoljem, kar bi lahko bilo oteževalno za vezavno teh struktur na predvidena receptorska mesta.