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Both 5-Arylidene-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-diones and 3-Thioxo-2,3-dihydro-1*H*-imidazo[1,5-*a*]indol-1-ones are Light-Dependent Tumor Necrosis Factor-α Antagonists

Matthew E. Voss,[†] Percy H. Carter,*[,†] Andrew J. Tebben, Peggy A. Scherle, Gregory D. Brown, Lorin A. Thompson, Meizhong Xu, Yvonne C. Lo, Gengjie Yang, Rui-Qin Liu, Paul Strzemienski, J. Gerry Everlof, James M. Trzaskos and Carl P. Decicco

Bristol-Myers Squibb Pharmaceuticals, Experimental Station, Rt. 141 & Henry Clay Road, Wilmington, DE 19880-0500, USA

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Abstract—Based on the realization that *N*-alkyl 5-arylidene-2-thioxo-1,3-thiazolidin-4-ones are tumor necrosis factor-α antagonists, we discovered two additional classes of antagonists: 3-thioxo-2,3-dihydro-1*H*-imidazo[1,5-*a*]indol-1-ones (via rational design) and 5-arylidene-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-diones (via computer-guided screening). Chemical modification of the lead structures showed that the structure–activity relationship profiles for both of these series were dependent on the electronic properties of the molecules. Subsequent studies showed that they were light-dependent inhibitors.

The clinical success of biological agents that sequester tumor necrosis factor alpha (TNF-α) in treating rheumatoid arthritis has highlighted the central role that this cytokine plays in the inflammatory response.[1,2] While several small molecules that block the production of soluble TNF-α are in clinical development,[3] the only potent inhibitors of the interaction between TNF-α and its cognate receptors are other proteins (antibodies and soluble receptors).[4] Given that the TNF-α/TNF Receptor-1 (TNFRc1) binding event capitalizes on avidity effects and also utilizes a large surface area for interaction,[5] the ineffectiveness of small organic compounds in blocking this interaction is not surprising. We recently reported that *N*-alkyl 5-arylidene-2-thioxo-1,3-thiazolidin-4-ones and 5-thioxo-5,6-dihydro-7*H*-thieno[2′,3′:4,5]pyrrolo[1,2-*c*]imidazol-7-ones (Fig. 1) are potent and selective inhibitors of the TNF-α/TNFRc1 interaction.[6] Herein we describe the structure–activity relationships (SARs) of two additional classes of TNF-α antagonists, the 3-thioxo-2,3-dihydro-1*H*-imidazo[1,5-*a*]indol-1-ones and 5-arylidene-2-thioxodihydro-pyrimidine-4,6(1*H*, 5*H*)-diones (Fig. 1).

*Corresponding author. Tel.: +1-302-467-6060; fax: +1-302-467-6910; e-mail: percy.carter@bms.com (P. H. Carter).
[†]Both authors contributed equally to this work.

5-Arylidene-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-diones

We used 5-arylidene-2-thioxo-1,3-thiazolidin-4-ones as lead compounds for a computer-driven similarity-based search of the DuPont Pharmaceuticals compound library. Screening of the thus-selected 20,000 compounds in the Eu^{3+}-TNF-α/TNFRc1 binding assay led to the identification of compounds **2**–**4** (Table 1), and thereby established 2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-diones as leads. Notably, replacement of the thiocarbonyl of **2** with a carbonyl eliminated its binding potency (0% inhibition at 10 μM), as expected based on the developing SAR of the 5-arylidene-2-thioxo-1,3-thiazolidin-4-one series.[6] Subsequent studies revealed that the SAR of compounds containing the 2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione head group and those containing the 2-thioxo-1,3-thiazolidin-4-one headgroup were different (Table 1). The indol-3-yl group was not a good arylidene substituent when paired with the 2-thioxo-1,3-thiazolidin-4-one headgroup, especially when compared to the 5-aryl-furan-2-yl and 5-aryl-thiophen-2-yl substituents (cf. **1**, **7**, **10**, and **13**). In contrast, indol-3-yl was >2-fold *better* than the 5-aryl-furan-2-yl and 5-aryl-thiophen-2-yl substiuents (cf. **5**, **8**, **11**, and **14**) in the 2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione series. Relative to their *N*,*N*-diaryl counterparts,

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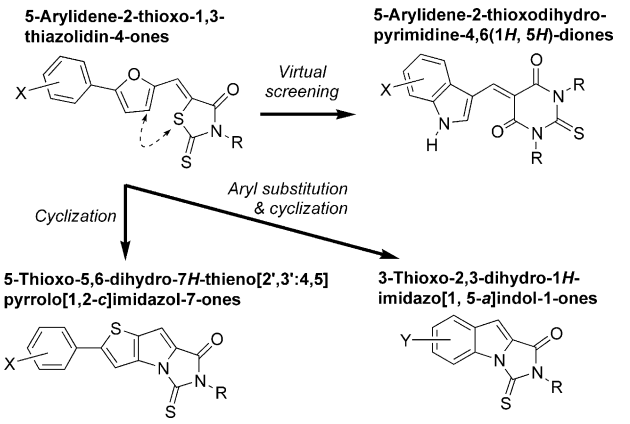


Figure 1. Four classes of TNF-α antagonists.

compounds with the *N*,*N*-diethyl 2-thioxodihydropyrimidine-4,6 (1*H*, 5*H*)-dione head group were equipotent (Table 1) and at least 3- to 20-fold less toxic (cf. **3**, $TC_{50} = 14$ μM and **5**, $TC_{50} > 100$ μM).[7] Accordingly, the remainder of our study utilized the *N*,*N*-diethyl heterocycle.[8]

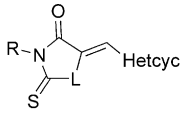
We next focused on fleshing out the SAR profile of the arylidene moiety (Table 2). The indol-3-yl group provided >10-fold more potency than the indol-2-yl group, any quinolinyl group, and any pyridinyl group (cf. **5**, **15**–**20**). Substitutions on the indole group were tolerated, so long as they did not make the ring too electron-deficient (cf. **21**–**23**, **24**–**25**). We observed a similar trend in the thiophen-3-yl series (cf. **26**–**27**, **28**–**30**). Collectively, the data in Tables 1 and 2 suggest that the electronic character of the arylidene group influences the potency of inhibitor binding,[9] and that optimal inhibition was obtained when the arylidene group was electron-rich.

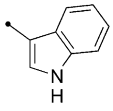
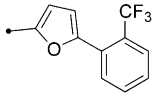
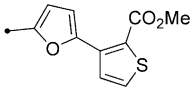
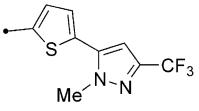
3-Thioxo-2,3-dihydro-1*H*-imidazo[1,5-*a*]indol-1-ones

The 3-thioxo-2,3-dihydro-1*H*-imidazo[1,5-*a*]indol-1-ones were designed based on the assumption that cyclization of the arylidene substituent and heterocyclic head group of the 5-arylidene-2-thioxo-1,3-thiazolidin-4-one should provide more potent inhibitors (Fig. 1). Some representative compounds are shown in Tables 3 and 4. The dependency of binding potency on the electronics of these inhibitors is immediately evident: as the electron-donating groups that are conjugated with the thiohydantoin carbonyl are increased, the potency of the TNF-α inhibition also increases (cf. **31**, **32**, **35**, and **37**, Table 3). The steric constraints on the inhibitors were not as evident; in particular, it appeared that the 6-alkoxy substituent and the heterocyclic *N*-substituent could be modified in a variety of ways (cf. **37** to **39**–**45**, Table 3). Furthermore, the indole nucleus could be methylated without dramatic effect (Table 4).

In order to probe further the SAR of the 3-thioxo-2,3-dihydro-1*H*-imidazo[1,5-*a*]indol-1-ones, modifications to the 'thiohydantoin region' were made (Table 4). As expected based on the work with the 2-thioxo-1,3-thiazolidin-4-ones[6] and 2-thioxodihydropyrimidine-

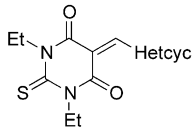
Table 1. Direct comparison of rhodanine-coupled (L = S) and 2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione-coupled (L = –NRC=O) heterocycles in TNFRc1 binding assay



Compd	L	R	Heterocycle	TNFRc1 Bnd, IC_{50}, μM[a]
1	–S–	Et		40% @ 10 μM
2	–N(R)C(O)–	Me		13.0
3	–N(R)C(O)–	*o*-Tolyl		0.79 (±0.02)
4	–NHC(O)–	*o*-Tolyl[b]		1.8 (±0.71)
5	–N(R)C(O)–	Et		1.0 (±0.39)
6	–N(R)C(O)–	Ph		9% @ 10 μM
7	–S–	Et		0.32 (±0.15)
8	–N(R)C(O)–	Et		2.0 (±0.57)
9	–N(R)C(O)–	Ph		11.0 (±3.1)
10	–S–	Et		0.3 (±0.23)
11	–N(R)C(O)–	Et		2.8 (±0.85)
12	–N(R)C(O)–	Ph		~40
13	–S–	Et		0.14 (±0.02)
14	–N(R)C(O)–	Et		33% @ 10 μM

[a]Inhibition of TNF-α binding (cf. ref 6) by the indicated compound at a concentration of 10 μM is listed as 'XX% @ 10'. The IC_{50} for binding (in μM) and standard deviation is given where available ($n \geq 2$).
[b]Olefin geometry not determined for this unsymmetrical thiobarbituite.

Table 2. Investigation of the TNFRc binding of 2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-diones reveals a dependence on the electronic properties of the inhibitors



Compd	Heterocycle	TNFRc1 Bnd, IC_{50}, μM[a]
5	Indol-3-yl	1.0 (±0.39)
15	Indol-2-yl	10.2 (±4.7)
16	Quinolin-3-yl	0% @ 10 μM
17	Quinolin-2-yl	0% @ 10 μM
18	Pyridin-3-yl	0% @ 10 μM
19	Pyridin-2-yl	0% @ 10 μM
20	Pyridin-4-yl	0% @ 10 μM
21	1-Me-indol-3-yl	0.13 (±0.03)
22	7-Me-indol-3-yl	0.6 (±0.28)
23	5-BnO-indol-3-yl	0.4 (±0.14)
24	5-NO_2-indol-3-yl	5.5 (±1.8)
25	1-Ac-indol-3-yl	15% @ 10 μM
26	5-Me-thiophen-2-yl	3.4 (±1.4)
27	5-Br-thiophen-2-yl	4.5 (±0.07)
28	4-Br-thiophen-2-yl	2% @ 10 μM
29	4-NO_2-thiophen-2-yl	0% @ 10 μM
30	3-Me-thiophen-2-yl	14.1 (±0.7)

[a]Inhibition of TNF-α binding (cf. ref 6) by the indicated compound at a concentration of 10 μM is listed as 'XX% @ 10'. The IC_{50} for binding (in μM) and standard deviation are given where available ($n \geq 2$).

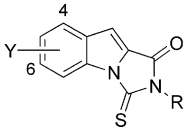
4,6(1*H*,5*H*)-diones (vide supra), replacement of the thiocarbonyl (C=S) with a carbonyl (C=O) (**46** and **49**) or imine (C=N–CN) (**50**) eliminated the biological activity of the 3-thioxo-2,3-dihydro-1*H*-imidazo[1,5-*a*]indol-1-ones. In this context, it was somewhat surprising that the positioning of the carbonyl and thiocarbonyl could be exchanged without large effect (cf. **37**/**47** and **48**/**51,** Table 4), and the bisthiocarbonyl analogue maintained equivalent activity (cf. **52** vs **48**, **51**). These results may speak to an element of pseudo-symmetry in these inhibitors. Modifications that disrupted the conjugation of the π-system—reduction (**53**) and ring expansion (**54**)—destroyed the binding activity of the compounds.

Given the novelty of the chemical structures of most of the compounds in Tables 3 and 4, a brief synthetic discussion is warranted (Scheme 1). The majority of the 3-thioxo-2,3-dihydro-1*H*-imidazo[1,5-*a*]indol-1-ones (**57**) can be prepared in moderate to low yields by heating the corresponding 2-indolecarboxylate (**55**),[10] an isothiocyanate, and triethylamine in a sealed tube at 135 °C.[11] If this reaction fails, the 2-indolecarboxylate can be reduced with Mg/methanol[12] to give the more reactive 2-indolinecarboxylate, which upon treatment with the isothiocyanate forms **56**. Air oxidation of **56** then provides an alternative and scalable route to **57**. A number of key analogues of the 3-thioxo-2,3-dihydro-1*H*-imidazo[1,5-*a*]indol-1-ones can be made from the amide **59** (Scheme 1), which is readily prepared from the corresponding 2-indolecarboxylic acid **58**. Thus, treatment of **59** with phosgene provides the imidazoindol-1,3-dione **60**, which is transformed into the 1-thioimidazoindol-3-one **61** by refluxing with Lawessen's reagent in toluene.[13] Alternatively, **59** can be reacted with diphenyl cyanocarbonimidate to give the cyanoguanidine **62**. Finally, the piperazinedione **63** was readily obtained from acid **58**.

Discussion of Biological Activity

Like our other inhibitors,[6] the majority of the 5-arylidene-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-diones and 3-thioxo-2,3-dihydro-1*H*-imidazo[1,5-*a*]indol-1-ones did not show any significant binding to CD40,[14] a closely

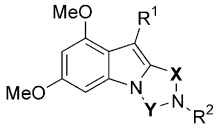
Table 3. Investigation of the TNFRc binding of 3-thioxo-2,3-dihydro-1*H*-imidazo[1,5-*a*]indol-1-ones reveals a dependence on the electronic properties of the inhibitors



Compd	Y	R	TNFRc1 Bnd, IC_{50}, μM[a]
31	H	Ethyl	27% @ 10 μM
32	4-Methoxy	Allyl	2.1
33	5-Chloro	Allyl	17% @ 10 μM
34	5-Benzyloxy	Allyl	46% @ 10 μM
35	6-Methoxy	Allyl	1.7 (±0.7)
36	5,6-Dimethoxy	Allyl	53% @ 10 μM
37	4,6-Dimethoxy	Allyl	0.28 (±0.11)
38	4,5,6-Trimethoxy	Allyl	0.78 (±0.32)
39	4,6-Dimethoxy	Ethyl	1.0 (±0.2)
40	4,6-Dimethoxy	–CH_2CH_2Ph	1.9 (±1.9)
41	4,6-Dimethoxy	4-Morpholinopropyl	0.67
42	4-MeO, 6-$MeOCH_2O$	Ethyl	0.28
43	4-MeO, 6-$tBuO_2CCH_2O$	Ethyl	0.39
44	4-MeO, 6-HO_2CCH_2O	Ethyl	42% @ 10 μM
45	4-MeO, 6-(2-Pyr)CH_2O[b]	Ethyl	0.31

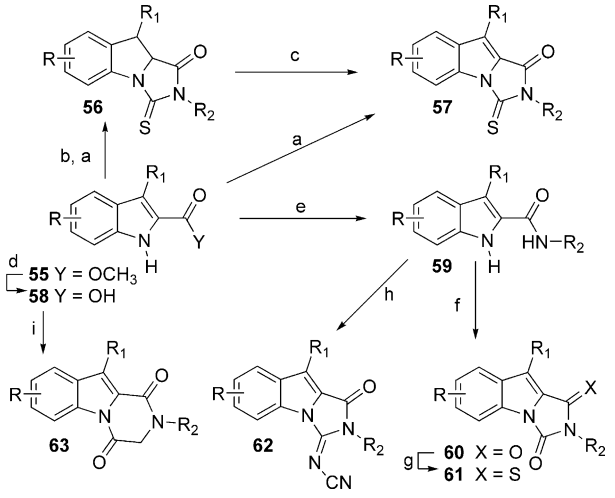
[a]Inhibition of TNF-α binding (cf. ref 6) by the indicated compound at a concentration of 10 μM is listed as 'XX% @ 10'. The IC_{50} for binding (in μM) and standard deviation is given where available ($n \geq 2$).
[b]Pyr = pyridinyl; the 3- and 4-pyridinyl compounds showed equivalent activity.

Table 4. Systematic structural modification of the thiohydantoin portion of the 3-thioxo-2,3-dihydro-1*H*-imidazo[1,5-*a*]indol-1-ones



Compd	R1	X	Y	R2	TNFRc1 Bnd, IC_{50}, μM[a]
37	H	C=O	C=S	Allyl	0.28 (±0.11)
46	H	C=O	C=O	Allyl	0% @ 10 μM
47	H	C=S	C=O	Allyl	0.61 (±0.43)
48	Me	C=O	C=S	Allyl	1.1
49	Me	C=O	C=O	Allyl	0% @ 10 μM
50	Me	C=O	C=N–CN	Allyl	19% @10 μM
51	Me	C=S	C=O	Allyl	0.21 (±0.06)
52	Me	C=S	C=S	Allyl	0.21
53	H_2[b]	C=O	C=S	Ethyl	0% @ 10 μM
54	Me	C=O	$C(=O)CH_2$	Benzyl	0% @ 10 μM

[a]Inhibition of TNF-α binding (cf. ref 6) by the indicated compound at a concentration of 10 μM is listed as 'XX% @ 10'. The IC_{50} for binding (in μM) and standard deviation is given where available ($n \geq 2$).
[b]The indole olefin was reduced (see Scheme 1).



Scheme 1. Synthesis of some key indolehydantoins. Reagents and conditions: (a) R_2-N=C=S (3 equiv), DIEA (2 equiv), 100–135 °C; (b) Mg, MeOH; (c) air oxidation; (d) KOH, MeOH; (e) R_2NH_2, BOP reagent, DIEA; (f) phosgene, DIEA, THF; (g) Lawessen's reagent; (h) $(PhO)_2C$=NCN, THF; (i) R_2NH-Gly-OEt, BOP reagent, DIEA.

related TNFRc1 homologue. Several of compounds from each series — for example, compounds **3**, **5**, **21**, **37**, **42**, and **47** — were also tested for their ability to block TNF-α-induced phosphorylation of Iκ-B in RAMOS cells, and exhibited potencies that ranged from 3 to 30 μM in this assay. In general, the potencies in this assay were weaker than those observed in the plate binding assay; it should be noted that a similar shift had been observed with our other inhibitors.[6] We assume that this difference in potency relates to the differential aggregation state of the TNFRc1 under the two assay conditions, an assumption that is partially validated by the closer correspondence in potency between the Iκ-B phosphorylation assay and a binding assay conducted using membranes from a CHO cell line that over-expresses a mutant TNFRc1.[15] The following data for plate binding, IkB inhibition, and membrane binding, are illustrative: 0.28, 11, 4.8 μM (**37**); 0.61, 10, 10 μM (**47**); and 0.28, 13, and 8.5 μM (**42**).

As described above, the SAR profiles for both the 5-arylidene-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-diones (Table 2) and 3-thioxo-2,3-dihydro-1*H*-imidazo[1,5-*a*]indol-1-ones (Tables 3, 4) suggested that, like in the 5-arylidene-2-thioxo-1,3-thiazolidin-4-one series, it was the electronics of the α, β-unsaturated amide carbonyl that were important. *Indeed, when the binding experiments were conducted in the dark, none of the tested 5-arylidene-2-thioxodihydropyrimidine-4,6(1*H,5H*)-diones or 3-thioxo-2,3-dihydro-1*H-*imidazo[1,5-*a*]indol-1-ones exhibited any inhibitory potency (data not shown).* Moreover, we found that the binding of both 5-arylidene-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-diones and 3-thioxo-2,3-dihydro-1*H*-imidazo[1,5-*a*]indol-1-ones was *irreversible* in the light.

In coincident work, we demonstrated that certain *N*-alkyl 5-arylidene-2-thioxo-1,3-thiazolidin-4-ones and 5-thioxo-5,6-dihydro-7*H*-thieno[2,3:4,5]pyrrolo[1,2-*c*]imidazol-7-ones associate weakly *and reversibly* with TNFRc1 in the dark, but undergo an irreversible photochemical reaction in the light.[6] Since the compounds are not reactive in solution, it appears that some aspect of the binding event (molecular strain, availability of electron transfer, etc.) allows the photochemical event to occur. In the present study, we were unable to verify that this same 'photochemically enhanced' binding was occuring, since we could not demonstrate any measurable binding of either the 5-arylidene-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-diones or 3-thioxo-2,3-dihydro-1*H*-imidazo[1,5-*a*]indol-1-ones in the absence of light. This may indicate that these inhibitors have lower inherent affinity for the TNFRc1,[16] or that a change in mechanism has occurred such that the photochemical event precedes binding. We assume that the binding is specific, however, because the compounds do not bind with high affinity to CD40 (vide supra)[14] and they do not react with simple amino acids in solution (not shown).

Regardless of the ordering of events, it is clear that the *apparent affinity* of these inhibitors for the TNFRc1 in

Table 5. Correlation of ΔHOMO–LUMO with compound potency

Compd	Compd class[a]	Δ(ΔHOMO–LUMO), kcal/mol[a]	TNFRc1 Bnd, IC_{50}, μM[b]
21	A	0	0.13 (±0.03)
5	A	+1.6	1.0 (±0.39)
26	A	+2.8	3.4 (±1.4)
25	A	+3.8	15% @ 10 μM
18	A	+12.1	0% @ 10 μM
16	A	−0.3	0% @ 10 μM
37	B	0	0.28 (±0.11)
47	B	−9.7	0.61 (±0.43)
35	B	+4.3	1.7 (±0.7)
31	B	+9.8	27% @ 10 μM
46	B	+17.1	0% @ 10 μM
53	B	+42.1	0% @ 10 μM

[a]Compounds in class A [the 5-arylidene-2-thioxodihydropyrimidine-4,6(1H, 5H)-diones] are referenced to **21** (ΔHOMO–LUMO = 209.1 kcal/mol, see ref 18). Compounds in class B (the 3-thioxo-2,3-dihydro-1H-imidazo[1,5-*a*]indol-1-ones) are referenced to **37** (ΔHOMO–LUMO = 209.8 kcal/mol).

[b]Inhibition of TNF-α binding (cf. ref 6) by the indicated compound at a concentration of 10 μM is listed as 'XX% @ 10'. The IC_{50} for binding (in μM) and standard deviation is given where available.

the light will be derived from several factors: (1) the inherent affinity of either the excited or unexcited state of the inhibitor for the protein; (2) the ability of the inhibitor, either bound or unbound to protein, to reach the excited state; and (3) the ability of chemical residues within the protein's binding pocket to promote covalent binding to the excited state. Since we did not have any data that allow us to assess the impact of the first or third factors on the binding of 5-arylidene-2-thioxo-dihydropyrimidine-4,6(1*H*,5*H*)-diones or 3-thioxo-2,3-dihydro-1*H*-imidazo[1,5-*a*]indol-1-ones, we moved to assess the inherent susceptibility towards photochemical excitation[17] through an estimation of the size of the gap between the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) of the inhibitors (Table 5).[18] The trend in ΔHOMO–LUMO energies generally correlates with the binding affinities, with one clear exception (**16**).[19] Importantly, both the measured binding affinity and the absolute calculated HOMO–LUMO gap for the reference 5-arylidene-2-thioxodihydro-pyrimidine-4,6(1*H*,5*H*)-dione (**21**, IC_{50} = 0.13 μM, ΔHOMO–LUMO = 209.1 kcal/mol) were similar to those of the reference 3-thioxo-2,3-dihydro-1*H*-imidazo[1,5-*a*]indol-1-one (**37**, IC_{50} = 0.28 μM, ΔHOMO–LUMO = 209.8 kcal/mol). Moreover, a brief study of UV spectral data of the 5-arylidene-2-thioxodihydro-pyrimidine-4,6(1*H*,5*H*)-diones **5**, **21**, and **25** was consistent with the trend predicted by the ΔHOMO–LUMO calculations.[9] Thus, the calculations do have some predictive value, even though the calculated HOMO–LUMO energies only provide an estimate of the energy required for the initial excitation in vacuum, and do not provide estimates of the energy of photoexcitation when bound to protein, the inherent solution affinity of the compound for the protein, or the chemical reactivity of the compound's excited state with the protein.[19]

Compounds similar to those described herein continue to appear in patent applications as leads for protein–protein interactions,[6,20] and we feel that it is important that the medicinal chemistry community be aware of the potential for the light-dependence of inhibitor binding with these and structurally-related compound classes.

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References and Notes

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7. Similar trends were observed with other direct comparisons (*N*,*N*-diaryl, Tox <25 μM; *N*,*N*-dialkyl, Tox >60 μM). The MTS toxicity assay was conducted in RAMOS cells (cf. ref 6 for protocol).
8. The synthesis of **21** exemplifies the easy synthesis of these analogues. To a solution of 1,3-diethyl-2-thiobarbituric acid (200 mg) in 10 mL of AcOH was added 240 mg of NaOAc and 159 mg of 1-methylindole-3-carboxaldehyde. The reaction was stirred for 14 h at rt and diluted with 50 mL of H_2O. The resultant precipitate was collected, washed (H_2O), and dried to afford 275 mg (81% yield) of **21** as a yellow solid: mp 235–236 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.63 (s, 1H), 9.03 (s, 1H), 8.02–7.80 (m, 1H), 7.45–7.38 (m, 3H), 4.63 (app quin, *J* = 6.6 Hz, 4H), 3.99 (s, 3H), 1.36 (app q, *J* = 6.7 Hz, 6H); IR (KBr) 1680, 1647, 1552, 1506 cm^{-1}; HRMS calcd for $C_{18}H_{20}N_3O_2S$ $(M+H)^+$: 342.1276; found: 342.1270.
9. A limited study of the UV spectra of the inhibitors **21**, **5**, and **25** (IC_{50}'s = 0.15, 1.0, and >10 μM, respectively) revealed that increasing potency was accompanied by a red shifting of the major peak. Furthermore, the absorption at 349 nM was not present in the spectrum of **25**, became evident in the spectrum of **5**, and was a strong absorption in the spectrum of **21**, suggesting that the intensity of this absorption was also correlated with increasing potency.
10. Generally, the 2-indolecarboxylates were purchased; in the case of analogues **42** through **45**, they were synthesized from 4-hydroxy-2-methoxybenzaldehyde using literature methods (see: Knittel, D. *Synthesis* **1985**, 186).
11. The synthesis of **41** is illustrative. Methyl 4,6-dimethoxy-2-indole carboxylate (0.25 g), *i*-Pr_2NEt (124 mg) and 3-(4-

morpholino)propyl isothiocyanate (0.59 g) were combined and heated in a sealed tube at 135 °C for 3 h. The mixture was cooled and the product was isolated (SiO_2, 20% EA/hex) and then recrystallized (EA/hex) to provide **41** (56 mg, 14% yield) as a yellow solid.

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14. The most potent TNFRc1 binders from each series were >100-fold less potent inhibitors of CD40: **21**, CD40 $IC_{50} = 48$ μM; **37**, CD40 $IC_{50} > 50$ μM.

15. (a) The TNFRc1 is fixed in the normal binding assay (presumably as a mixture of aggregation states), whereas it is free to associate and dissociate in the other two assays. The importance of aggregation for TNF-α binding has been discussed (see ref 5). (b) For the assay, see ref 6. For the C-terminally truncated TNFRc1, see: Brakebusch, C.; Nophar, Y.; Kemper, O.; Engelmann, H.; Wallach, D. *EMBO J.* **1992**, *11*, 943.

16. It is not clear whether or not the 5-arylidene-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-diones and 3-thioxo-2,3-dihydro-1*H*-imidazo[1,5-*a*]indol-1-ones interact with the Ala62 region of the TNFRc1 in a similar fashion to the 5-arylidene-2-thioxo-1,3-thiazolidin-4-ones and 5-thioxo-5,6-dihydro-7*H*-thieno[2′,3′:4,5]pyrrolo[1,2-*c*]imidazol-7-ones (see ref 6). Given the differences in their binding behavior in the dark (see text), their obvious structural differences (Fig. 1), and their differences in SAR (Table 1), it may well be that these inhibitors bind to a different pocket, or to the same pocket in a different mode.

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18. The HOMO–LUMO gap energies were generated from MP2/6-311G** single point energy calculations on HF/6-31G* optimized geometries of the inhibitors. The program utilized was Jaguar 4.2 (Schrodinger, Inc., Portland, OR, USA).

19. (a) The result with **16** may arise because of a lower *inherent* affinity of *basic* heterocycles for the protein. (b) The calculations agreed with the difference in activity associated with the subtle structural difference between **27** and **28**: $\Delta(\Delta\text{HOMO–LUMO}) = +3.66$ kcal/mol (HF/LACV3P** basis set). (c) Some of the *absolute* calculated values vary from what one might expect (**35** and **47**).

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