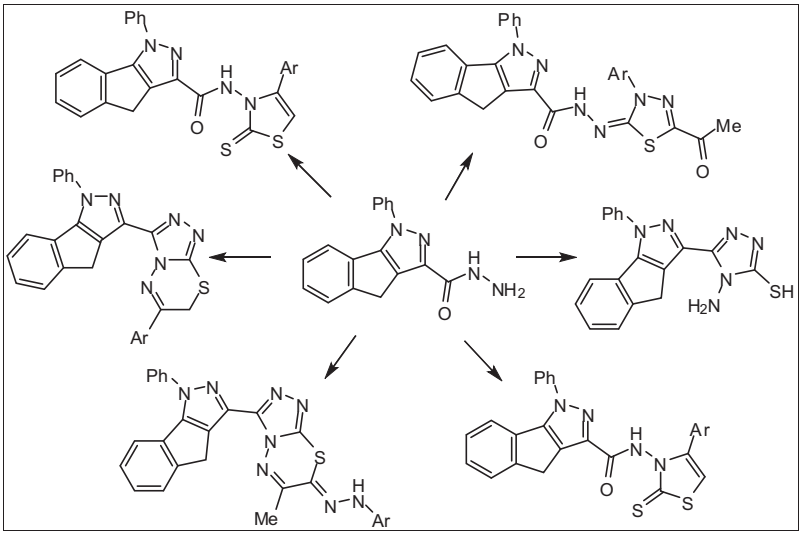
Bahira Hegazi,[a] Hassan Abdel-Gawad,[a] Hanan A. Mohamed,[a] Farid A. Badria,[b] and Ahmad M. Farag[c]*

[a]Department of Applied Organic Chemistry, National Research Centre, Dokki, Giza 12622, Egypt
[b]Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt
[c]Department of Chemistry, Faculty of Science, Cairo University, Giza 12613, Egypt
*E-mail: afarag49@yahoo.com

Received June 17, 2011
DOI 10.1002/jhet.1566
Published online 20 March 2013 in Wiley Online Library (wileyonlinelibrary.com).



Reaction of potassium 2-(1-phenyl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carbonyl)hydrazine carbodithioate (**2**) with hydrazine hydrate, phenacyl bromides, and hydrazonoyl chlorides afforded the corresponding 1,2,4-triazole, 1,3-thiazole, and 1,3,4-thiadiazole derivatives **3**, **5**, and **7**, respectively. Reaction of 3-mercapto-4-amino-1,2,4-triazole **3** with phenacyl bromides **4a–c** and hydrazonoyl chlorides **6a,b** afforded the corresponding 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine derivatives **10a–c** and **13a,b**, respectively, whereas its reaction with 2-chloro-*N*-arylacetamide derivatives **11a–d** afforded the corresponding 2-(4-amino-5-(1-phenyl-1,4-dihydroindeno[1,2-*c*]pyrazol-3-yl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-arylacetamide derivatives **12a–d**. All of the new compounds were tested for their protection activity against DNA damage induced by bleomycin–iron complex. Compounds **7a** and **b** showed the highest protection activity through diminishing chromogen formation between the damaged DNA and thiobarbituric acid.

J. Heterocyclic Chem., **50**, 355 (2013).

INTRODUCTION

Acid hydrazides have been in the center of attention of researchers over many years because of the high practical value of these compounds. In the first place, acid hydrazides are very important class of compounds, for their high reactivity in heterocyclic synthesis, as key starting materials to form various classes of biologically and pharmacologically active candidates [1–5]. For example, 1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives are as fungicides [6] and antitumor agents [7]. In addition, substituted hydrazides are characterized by low toxicity and possess a broad spectrum of pharmacological activity, including antibacterial, anthelmintic, fungicidal, antiviral, antitumor, hypoglycemic, hypolipidemic, antisclerotic, antiaggregant, antithrombin, hemostatic, hypertensive, antiarrhythmic, antidepressant, anticonvulsant, antiinflammatory, analgesic, antihypoxant, biligenic, hepatoprotector, and diuretic effects [8]. In view of these facts and in continuation of our interest in the synthesis of a variety of heterocycles of biological importance [9–30], we report here an efficient and convenient method for the synthesis of novel 1,3-thiazole, 1,3,4-thiadiazole, 1,2,4-triazole, and 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives attached to 1,4-dihydro-1-phenylindeno[1,2-*c*] pyrazole moiety. The synthesized compounds were evaluated for their protection activities against DNA damage induced by bleomycin–iron assay, and this may help to reduce the side effects of chemotherapy for cancer patients.

RESULTS AND DISCUSSION

Treatment of the acid hydrazide **1** [20], with carbon disulfide, in the presence of potassium hydroxide, resulted

in the formation of the potassium salt of carbodithioate **2**. Treatment of the salt **2** with hydrazine hydrate in aqueous ethanol afforded the corresponding 4-amino-1,2,4-triazole-5-thione **3**, which exists also in the tautomeric thiol form **3a**. When the potassium salt **2** was treated with phenacyl bromide derivatives **4a–c**, it gave the corresponding 1, 3-thiazole derivatives **5**, whereas its reaction with the hydrazonoyl chlorides **6a,b** viz. 1-(2-phenylhydrazono)-1-chloropropan-2-one (**6a**) and 1-(2-(4-fluorophenyl)-hydrazono)-1-chloropropan-2-one (**6b**), under the same conditions, produced the corresponding 1,3,4-thiadiazole derivatives **7a** and **7b**, respectively (Scheme 1).

The structures of compounds **3**, **5a–c**, and **7a,b** are in complete agreement with the spectral and analytical data. For example, the ^{1}H NMR spectrum of compound **5a** revealed a singlet at δ 8.11 attributed to CH of the 2-thioxothiazole ring. The mass spectrum of the products **3**, **5c**, and **7b** revealed molecular ion peaks at *m*/*z* 346, 500, and 510, respectively.

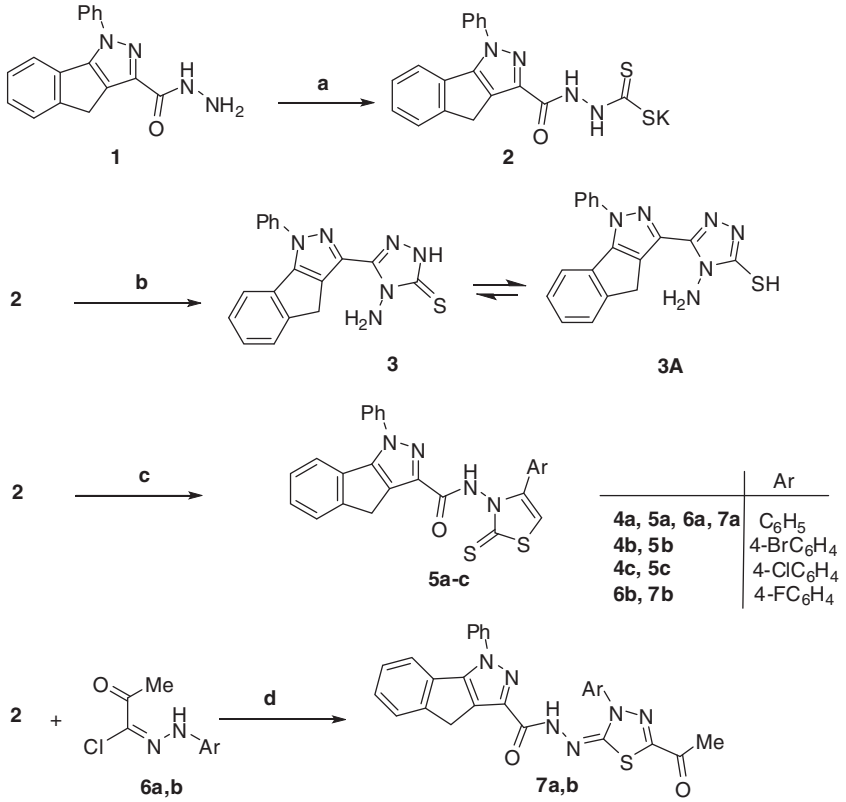
The Schiff base **8** was produced by the reaction of 4-amino-1,2,4-triazole-5-thione derivative **3** with 4-fluorobenzaldehyde under reflux in ethanol containing a few drops of glacial acetic acid as a catalyst. The reaction of compound **6** with the phenacyl bromide derivatives **4a–c** or hydrazonoyl chlorides **6a**,**b**, in ethanol containing a catalytic amount of triethylamine, furnished the corresponding 1,2,4-triazolo [3,4-*b*]-1,3,4-thiadiazine derivatives **10a–c** and **13a**,**b**, respectively (Scheme 2).

The reaction of 2-chloro-*N*-arylacetamides **11a–d** with the 4-amino-1,2,4-triazole-5-thione **3**, under the same conditions, furnished the acetamide derivatives **12a–d** (Scheme 2). The structures of compounds **8**, **10a–c**, **12a–d**, and **13a**,**b** were established on the basis of both microanalytical and spectral data. For example, the ^{1}H NMR spectrum of compound **8** revealed a singlet signal at δ 8.72 attributed to –N=C<u>H</u>–, whereas the ^{1}H NMR spectrum of compound **10a** showed a singlet signal at 4.96 ppm corresponding to the two protons of-S-C<u>H</u>$_2$ in the thiadiazine ring. The mass spectra of compounds **12a** and **13b** revealed molecular ion peaks at *m*/*z* 497 and 506, respectively.

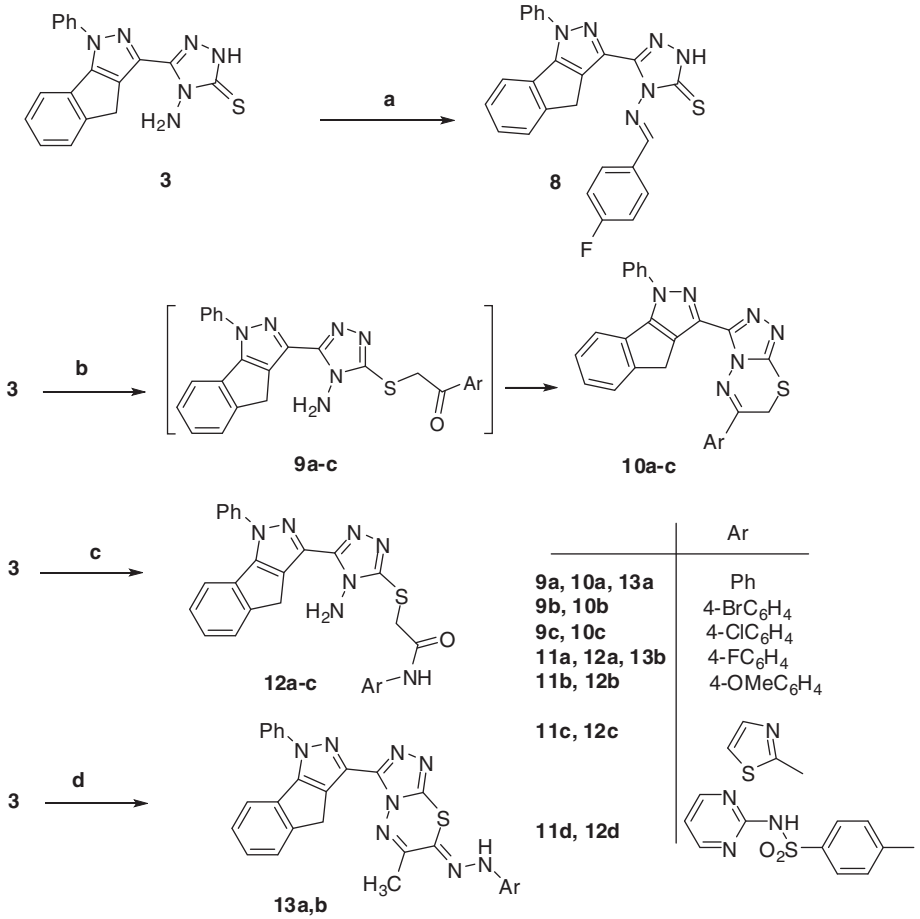
Bleomycin-dependent DNA damage. The bleomycins are a family of glycopeptide antibiotics that are used routinely as antitumor agents. The bleomycin assay has been adopted for assessing the pro-oxidant effects of food antioxidants. The antitumor antibiotic bleomycin binds iron ions and DNA. The bleomycin–iron complex degrades DNA that, upon heating with thiobarbituric acid (TBA), yields a pink chromogen. Added suitable reducing agents "antioxidants" compete with DNA and diminish chromogen formation [31].

All of the new synthesized compounds were tested for their protection activity against DNA damage induced by bleomycin–iron complex. Table 1 illustrates that compounds **7a** and **7b** showed the highest protection activity against DNA damage induced by bleomycin–iron complex thus diminishing chromogen formation between the damaged

Scheme 1. Reagents and condition: (a) CS_2/KOH/EtOH, 0°C, stirring 1 h; (b) $NH_2NH_2{\cdot}H_2O$; (c) $ArCOCH_2Br$ **4a–c**, EtOH, reflux; (d) EtOH, reflux.



Scheme 2. Reagent and conditions: (a) 4-FC_6H_4CHO, EtOH–AcOH, reflux; (b) $ArCOCH_2Br$ **4a–c**, EtOH/Et_3N; reflux; (c) $ArNHCOCH_2Cl$ **11a–c**, EtOH/Et3N; reflux; (d) **6a**,**b**, EtOH/Et_3N; reflux.



DNA and TBA. On the other hand, compounds **10a**, **10b**, and **12b** showed a very low activity, whereas the rest of the tested compounds exhibited a weak to moderate activity.

Structure activity relationship. Thiadiazole is a versatile moiety that exhibits a wide variety of biological activities. Thiadiazole moiety acts as "hydrogen binding domain" and "two-electron donor system". It also acts as a constrained pharmacophore. Many drugs containing thiadiazole nucleus are available in the market such as acetazolamide, methazolamide, sulfamethazole, and so on. Thiadiazole can act as the bioisosteric replacement of the thiazole moiety. So it acts like third and fourth generation cephalosporins, hence can be used in antibiotic preparations. Members of this ring system have found their way into such diverse application as pharmaceuticals, oxidation inhibitors, cyanine dyes, and metal complexing agents. Thiadiazole derivatives have antimicrobial, anti-inflammatory, anticancer, anti-convulsant, antidepressant, antioxidant, radio protective, and antileishmanial activities [32].

From the obtained results (Table 1), it can be concluded that 1,4-dihydro-1-phenylindeno[1,2-*c*]pyrazole moiety is essential for the protection activity against DNA damage induced by bleomycin–iron complex, and 1,3-thiazole ring is also required for the activity and an unsubstituted phenyl ring exhibited better activity than those substituted derivatives. This good activity of thiazole derivatives shows the hypothesis of a direct link between thiol function and an aromatic ring to be a good one. The thiol catches the radical, and after, the aromatic ring permits the trapping of this radical. Moreover, aminothiol derivative of thiadiazole shows a better activity.

CONCLUSION

All of the new synthesized compounds were tested for their protection activity against DNA damage induced by bleomycin–iron complex. Compounds **7a** and **7b** showed the highest protection activity against DNA damage. On the other hand, compounds **10a**, **10b**, and **12b** showed a very low activity, whereas the rest of the tested compounds exhibited weak to moderate activity.

Table 1

Results of bleomycin-dependent DNA damage assay of the synthesized compounds.

Sample (extract)	DNA absorbance (λ 532 nm)
Negative control[a]	—
Butylated hydroxyanisole (BHA)[b]	0.0026
Vitamin C (ascorbic acid)	0.00370
Compound **3**	0.05
5a	0.45
5b	0.18
5c	0.18
7a	0.004
7b	0.004
8	0.006
10a	0.28
10b	0.45
10c	0.24
12a	0.18
12b	0.50
12c	0.18
12d	0.18
13a	0.008
13b	0.05

[a]Negative control means no reaction mixture and no DNA absorbance.
[b]Butylated hydroxyanisole (BHA) is an antioxidant consisting of a mixture of 2-*tert*-butyl-4-hydroxyanisole and 3-*tert*-butyl-4-hydroxyanisole. The conjugated aromatic ring of BHA is able to stabilize free radicals. BHA and vitamin C (ascorbic acid) act as free radical scavengers.

It can be concluded that 1,4-dihydro-1-phenylindeno[1,2-*c*]pyrazole moiety is essential for the protection activity against DNA damage induced by bleomycin–iron complex, and 1,3-thiazole ring is also required for the activity. On the other hand, unsubstituted phenyl ring exhibited better activity than those substituted derivatives

The equipotent activity of compounds **7a** and **7b** confirms that the introduction of fluorine atom into aromatic ring (**7b**) does not enhance the activity. Interestingly, the introduction of Br atom in the aromatic system of compound **10b** reduced the activity in comparison with the chloro derivative in **10c** that retains almost the same activity of the nonsubstituted aromatic system (**10a**).

EXPERIMENTAL

General remarks. All melting points were taken on Electrothermal IA 9000 series digital melting point apparatus (Electrothermal House, Rochford, Great Britain). Elemental analytical data were carried from the microanalytical unit, Cairo University, Giza, Egypt. Electronic spectra were recorded using a Unicam UV2-100 UV/VIS spectrophotometer (Unicam Instruments Ltd., Cambridge, United Kingdom). The IR spectra were recorded in potassium bromide disks on a Shimadzu CVT-04 spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan). The ^{1}H NMR spectra were recorded at 270 MHz on a Varian EM-360 spectrometer (Varian, Inc., USA) and on a Bruker 400 MHz spectrometer (Bruker Scientific Instruments, Billerica, MA) using TMS as an internal standard. Chemical shift values (δ) are given in parts per million (ppm). The mass spectra were performed using a Varian MAT CH-5 mass spectrometer at 70 eV. 1,4-Dihydro-1-phenylindeno[1,2-*c*]pyrazole-3-carbohydrazide (**1**) [20], hydrazonoyl chlorides **6a** [33], **6b** [34], 2-chloro-*N*-arylacetamides **11a–d** [35–38] were prepared according to the procedures reported in literature.

***Potassium 2-(1-phenyl-1,4-dihydroindeno[1,2-c]pyrazole-3-carbonyl)hydrazine-carbodithioate* (2).** To a solution of the acid hydrazide **1** (2.9 g, 10 mmol) in ethanol (100 mL), potassium hydroxide solution (0.84 g, 15 mmol) in water (10 mL) and carbon disulfide (1.5 g, 20 mmol) were added. The reaction mixture was stirred in an ice bath for 3 h. The residue was then treated with dry benzene (30 mL), and the formed precipitate was collected by filtration, washed with ether, and dried to afford the potassium salt **2** in 85% yield. IR (KBr): ύ (cm^{-1}) 3,370, 3,133 (2NH), 1,652 (C═O).

***4-Amino-3-(1-phenyl-1,4-dihydroindeno[1,2-c]pyrazol-3-yl)-1H-1,2,4-triazole-5(4H)-thione* (3).** To a solution of **2** (4.04 g, 10 mmol) in ethanol (20 mL) and water (10 mL), hydrazine hydrate 80% (1 g, 20 mmol) was added. The reaction mixture was heated under reflux for 3 h, left to cool, and then poured onto crushed ice. The resulting solution was treated with dilute hydrochloric acid and adjusted to pH 7, and the resulting solid was collected by filtration, washed with water, dried, and finally recrystallized from ethanol to give 1,2,4-triazole-5-thione derivative **3**. Yield: 72%; mp 206–208°C; IR (KBr): ύ 1,282 (SH) 3,338, 3,118 (NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.83 (s, 2H, indane-3-CH_2), 7.19–7.61 (m, 9H, Ar–H), 9.68 (s, 2H, NH_2, D_2O-exchangeable), 12.36 (s, 1H, NH, D_2O-exchangeable); MS *m*/*z* (%) 346 (M^+, 26), 77 (100). *Anal*. Calcd for $C_{18}H_{14}N_6S$ (346.10): C, 62.41; H, 4.07; N, 24.26. Found: C, 62.49; H, 3.97; N, 24.34%.

***2-Thioxothiazol-3(2H)-yl)-1-phenyl-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxamides* (5a–c).** A mixture of **2** (0.81 g, 2 mmol) and appropriate phenacyl bromide **4** (2 mmol) in ethanol (20 mL) was heated under reflux for 3 h. The formed solid was collected by filtration, washed with ethanol, dried, and recrystallized from EtOH–DMF to afford **5a–c**.

***1-Phenyl-N-(4-phenyl-2-thioxothiazol-3(2H)-yl)-1,4-dihydroindeno[1,2-c]pyrazole-3 carboxamide* (5a).** Yield 65%; mp 167–168°C; IR (KBr): ύ (cm^{-1}) 1,584 (C═O), 3,298 (NH); ^{1}H NMR (DMSO-d_6): δ 3.77 (s, 2H, indane-3-CH_2), 7.36–8.00 (m, 14H, Ar–H), 8.11 (s, 1H, CH), 10.81 (s, 1H, NH, D_2O-exchangeable); MS *m*/*z* (%) 466 (M^+, 5.6), 77 (100). *Anal*. Calcd for $C_{26}H_{18}N_4OS_2$ (466.09): C, 66.93; H, 3.89; N, 12.01. Found: C, 66.99; H, 3.98; N, 11.89%.

***N*-(4-(4-Bromophenyl)-2-thioxothiazol-3(2*H*)-yl)-1-phenyl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide (5b)**. Yield 73%; mp 205–207°C; IR (KBr): ύ (cm^{-1}) 1,687 (C═O), 3,292 (NH); ^{1}H NMR (DMSO-*d6*): δ 3.86 (s, 2H, indane-3-CH_2), 7.38–7.80 (m, 13H, Ar–H), 8.02 (s, 1H, CH), 10.85 (s, 1H, NH, D_2O-exchangeable); MS *m*/*z* (%): 544, 546 (M^+, 3.86, 3.57), 77 (100). *Anal*. Calcd for $C_{26}H_{17}BrN_4OS_2$ (544.00): C, 57.25; H, 3.14; N, 10.27. Found: C, 57.36; H, 3.02; N, 10.11%.

***N*-(4-(4-Chlorophenyl)-2-thioxothiazol-3(2*H*)-yl)-1-phenyl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide (5c)**. Yield 68%; mp 215–216°C; IR (KBr): ύ (cm^{-1}) 1,682 (C═O), 3,295 (NH); ^{1}H NMR (DMSO-d_6): δ 3.84 (s, 2H, indane-3-CH_2), 7.38–7.95 (m, 13H, Ar–H), 8.00 (s, 1H, CH), 10.80 (s, 1H, NH, D_2O-exchangeable); MS *m*/*z* (%): 502 (M^++ 2, 3), 500 (M^+, 8.85), 77 (100). *Anal*. Calcd for $C_{26}H_{17}ClN_4OS_2$ (500.05): C, 62.33; H, 3.42; N, 11.18. Found: C, 62.41; H, 3.53; N, 11.24%.

***N′*-(5-Acetyl-3-aryl-1,3,4-thiadiazol-2(3*H*)-ylidene)-1-phenyl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carbohydrazide (7a,b)**. To a solution of **2** (0.81 g, 2 mmol) in ethanol (20 mL), the appropriate hydrazonyl chloride **6** (2 mmol) was added. The reaction mixture was heated under reflux for 3 h, then left to cool. The formed solid was collected by filtration, washed with ethanol, dried, and recrystallized from EtOH–DMF to afford **7a**,**b**.

***N′*-(5-Acetyl-3-phenyl-1,3,4-thiadiazol-2(3*H*)-ylidene)-1-phenyl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carbohydrazide (7a)**. Yield 67%; mp 195–196°C; IR (KBr): ύ (cm^{-1}) 1,652, 1,681 (2C═O), 3,195 (NH); ^{1}H NMR (DMSO-d_6): δ 2.42 (s, 3H, CH_3), 3.96 (s, 2H, indane-3-CH_2), 7.20–8.04 (m, 14H, Ar–H), 11.21 (s, 1H, NH, D_2O-exchangeable); MS *m*/*z* (%): 492 (M^+, 28), 77 (100). *Anal*. Calcd for $C_{27}H_{20}N_6O_2S$ (492.14): C, 65.84; H, 4.09; N, 17.06. Found: C, 65.91; H, 4.14; N, 17.21%.

***N′*-(5-Acetyl-3-phenyl-1,3,4-thiadiazol-2(3*H*)-ylidene)-1-(4-fluorophenyl)-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carbohydrazide (7b)**. Yield 72%; mp 198–199°C; IR (KBr): ύ (cm^{-1}) 1,656, 1,683 (2C═O), 3,196 (NH); ^{1}H NMR (DMSO-d_6): δ 2.43 (s, 3H, CH_3), 3.89 (s, 2H, indane-3-CH_2), 7.24–8.01 (m, 13H, Ar–H), 11.19 (s, 1H, NH, D_2O-exchangeable); MS *m*/*z* (%): 510 (M^+, 28), 259 (100). *Anal*. Calcd for $C_{27}H_{19}FN_6O_2S$ (510.13): C, 63.52; H, 3.75; N, 16.46. Found: C, 63.61; H, 3.62; N, 16.52%.

***4-(4-Fluorobenzylideneamino)-5-(1-phenyl-1,4-dihydroindeno[1,2-*c*]pyrazol-3-yl)-4*H*-1,2,4-triazole-3-thiol (8)**. A mixture of 4-fluorobenzaldehyde (0.25 g, 2 mmol) and **3** (0.69 g, 2 mmol) in ethanol (20 mL) and glacial acetic acid (0.2 mL) was heated under reflux for 4 h, then left to cool. The formed solid product was collected by filtration, washed with ethanol, and recrystallized from acetic acid. Yield 75%; mp 262–263°C; IR (KBr): ύ (cm^{-1}) 1,597 (C═N), 3,172 (NH); ^{1}H NMR (DMSO-d_6): δ 3.86 (s, 2H, indane-3-CH_2), 7.29–8.06 (m, 13H, Ar–H), 8.72 (s, 1H, CH═N), 11.85 (s, 1H, NH, D_2O-exchangeable), 13.95 (s, 1H, SH); MS *m*/*z* (%): 452 (M^+, 9.2), 77 (100). *Anal*. Calcd for $C_{25}H_{17}FN_6S$ (452.12): C, 66.36; H, 3.79; N, 18.57. Found: C, 66.43; H, 3.68; N, 18.65%.

General procedures for the synthesis of 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines 10a–c*, *13a*,*b*, and the acetamides 12a–d. A mixture of **3** (0.69 g, 2 mmol) and the appropriate phenacyl bromides **4a–c**, 2-chloro-*N*-arylacetamides **11a–d** or hydrazonyl chlorides **6a**,**b** (2 mmol) in absolute ethanol (30 mL) containing triethylamine (0.2 g, 2 mmol) was heated under reflux for 3 h, then left cool. The formed precipitate was isolated by filtration, washed with ethanol, dried, and finally recrystallized from EtOH–DMF to afford the products **10a–c**, **12a–d**, and **13a**,**b**, respectively.

***6-Phenyl-3-(1-phenyl-1,4-dihydroindeno[1,2-*c*]pyrazol-3-yl)-7*H*-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine (10a)**. Yield 61%; mp 220–222°C; ^{1}H NMR (DMSO-*d6*): δ 3.87 (s, 2H, indane-3-CH_2), 4.96 (s, 2H, S-CH_2), 7.38–8.11 (m, 14H, Ar–H); MS *m*/*z* (%): 446 (M^+, 8.5), 77 (100). *Anal*. Calcd for $C_{26}H_{18}N_6S$ (446.13): C, 69.94; H, 4.06; N, 18.82. Found: C, 70.11; H, 4.13; N, 18.91%.

***6-(4-Bromophenyl)-3-(1-phenyl-1,4-dihydroindeno[1,2-*c*]pyrazol-3-yl)-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine (10b)**. Yield 66%; mp 251–252°C; ^{1}H NMR (DMSO-d_6): δ 3.81 (s, 2H, indane-3-CH_2), 4.50 (s, 2H, S-CH_2), 7.39–8.10 (m, 13H, Ar–H); MS *m*/*z* (%): 526 (M^+, 11.27), 525 (M^+ + 1, 34.15), 524 (M^+ + 11.8), 77 (100). *Anal*. Calcd for $C_{26}H_{17}BrN_6S$ (524.04): C, 59.43; H, 3.26; N, 15.99. Found: C, 59.50; H, 3.19; N, 16.20%.

***6-(4-Chlorophenyl)-3-(1-phenyl-1,4-dihydroindeno[1,2-*c*]pyrazol-3-yl)-7*H*-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine (10c)**. Yield 74%; mp: 226–227°C; ^{1}H NMR (DMSO-d_6): δ 3.85 (s, 2H, indane-3-CH_2), 4.51 (s, 2H, S-CH_2), 7.37–8.13 (m, 13H, Ar–H); MS *m*/*z* (%): 482 (M^+ + 2, 4.1), (480 (M^+, 22.5), 77 (100). *Anal*. Calcd for $C_{26}H_{17}ClN_6S$ (480.09): C, 64.93; H, 3.56; N, 17.47. Found: C, 64.99; H, 3.61; N, 17.53%.

***2-(4-Amino-5-(1-phenyl-1,4-dihydroindeno[1,2-*c*]pyrazol-3-yl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-fluorophenyl)acetamide (12a)**. Yield 55%; mp 245–246°C; IR (KBr): ύ (cm^{-1}) 1631 (C═O), 3,330–3081 (NH_2, NH); ^{1}H NMR (DMSO-d_6): δ 3.88 (s, 2H, indane-3-CH_2), 4.35 (s, 2H, S-CH_2), 6.35 (s, 2H, NH_2, D_2O-exchangeable), 7.29–7.90 (m, 13H, Ar–H), 10.49 (s, 1H, NH, D_2O-exchangeable); MS *m*/*z* (%): 497 (M^+, 18.9), 77 (100). *Anal*. Calcd for $C_{26}H_{20}FN_7OS$ (497.14): C, 62.76; H, 4.05; N, 19.71. Found: C, 62.81; H, 3.99; N, 19.78%.

***2-(4-Amino-5-(1-phenyl-1,4-dihydroindeno[1,2-*c*]pyrazol-3-yl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-methoxyphenyl)acetamide (12b)**. Yield 57%; mp: 248–249°C; IR (KBr): ύ (cm^{-1}) 1627 (C═O), 3,326–3061 (NH_2, NH); ^{1}H NMR (DMSO-d_6): δ 3.91 (s, OCH_3), 3.80 (s, 2H, indane-3-CH_2), 4.19 (s, 2H, S-CH_2), 6.35 (s, 2H, NH_2, D_2O-exchangeable), 7.38–7.89 (m, 13H, Ar–H), 10.45 (s, 1H, NH, D_2O-exchangeable); MS *m*/*z* (%): 509 (M^+, 20.23), 259 (100). *Anal*. Calcd for $C_{27}H_{23}N_7O_2S$ (509.16): C, 63.64; H, 4.55; N, 19.24. Found: C, 63.66; H, 4.62; N, 19.35%.

***2-(4-Amino-5-(1-phenyl-1,4-dihydroindeno[1,2-c]pyrazol-3-yl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(thiazol-2-yl)acetamide (12c)**. Yield 61%; mp 276–277°C; IR (KBr): ύ (cm^{-1}) 1677 (C═O), 3,326–3068 (NH_2, NH); ^{1}H NMR (DMSO-d_6): δ 3.88 (s, 2H, indane-3-CH_2), 4.46 (s, 2H, S-CH_2), 6.35 (s, 2H, NH_2, D_2O-exchangeable), 7.25–7.88 (m, 11H, Ar–H), 12.42 (s, 1H, NH, D_2O-exchangeable); MS *m*/*z* (%): 486 (M^+, 18.9), 77 (100). *Anal*. Calcd for $C_{23}H_{18}N_8OS_2$ (486.10): C, 56.77; H, 3.73; N, 23.03. Found: C, 56.83; H, 3.79; N, 23.19%.

***2-(4-Amino-5-(1-phenyl-1,4-dihydroindeno[1,2-*c*]pyrazol-3-yl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-(*N′*-pyrimidin-2-ylsulfamoyl) phenyl)acetamide (12d)**. Yield 63%; mp 232–233°C; IR (KBr): ύ (cm^{-1}) 1628 (C═O), 3,332–3046 (NH_2, NH); ^{1}H NMR (DMSO-d_6): δ 3.87 (s, 2H, indane-3-CH_2), 4.23 (s, 2H, S-CH_2), 6.34 (s, 2H, NH_2, D_2O-exchangeable), 7.38–8.02 (m, 16H, Ar–H), 10.81, 11.33 (2s, 2H, 2NH, D_2O-exchangeable); MS *m*/*z* (%): 636 (M^+, 6.45), 60 (100). *Anal*. Calcd for $C_{30}H_{24}N_{10}O_3S_2$ (636.15): C, 56.59; H, 3.80; N, 22.00. Found: C, 57.63; H, 3.83; N, 22.31%.

***6-Methyl-3-(1-phenyl-1,4-dihydroindeno[1,2-*c*]pyrazol-3-yl)-7-(2-phenylhydrazono)-7*H*-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine (13a)**. Yield 53%; mp 240–241°C; ^{1}H NMR (DMSO-d_6): δ 2.51 (s, 3H, CH_3), 3.84 (s, 2H, indane-3-CH_2), 7.36–8.02 (m, 14H, Ar–H), 11.21 (s, 1H, NH, D_2O-exchangeable); MS *m*/*z* (%): 488 (M^+, 100). *Anal*. Calcd for $C_{27}H_{20}N_8S$ (488.15): C, 66.38; H, 4.13; N, 22.94. Found: C, 66.43; H, 4.25; N, 22.88%.

***7-(2-(4-Fluorophenyl)hydrazono)-6-methyl-3-(1-phenyl-1,4-dihydroindeno[1,2-*c*]pyrazol-3-yl)-7*H*-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine (13b)**. Yield 55%; mp 258–259°C; ^{1}H NMR (DMSO-d_6): δ 2.53 (s, 3H, CH_3), 3.86 (s, 2H, indane-3-CH_2), 7.37–8.09 (m, 13H, Ar–H), 11.24 (s, 1H, NH, D_2O-exchangeable); MS *m*/*z* (%): 506 (M^+, 100). *Anal*. Calcd for $C_{27}H_{19}FN_8S$ (506.14): C, 64.02; H, 3.78; N, 22.12. Found: C, 64.11; H, 3.82; N, 22.29%.

BLEOMYCIN-DEPENDENT DNA DAMAGE ASSAY [31,39,40]

The reaction mixtures are contained in a final volume of 1.0 mL; the following reagents at the final concentrations are stated: DNA (0.2 mg/mL), bleomycin (0.05 mg/mL), $FeCl_3$ (0.025 mM, 0.0067 mg/mL), $MgCl_2$ (5 mM, 0.47 mg/mL),

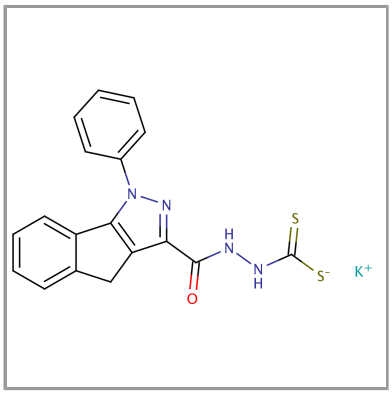
KH_2PO_4–KOH buffer pH 7.0 (30 mM), and ascorbic acid (0.24 mM, 0.042 mg/ml) or the fractions tested diluted in MeOH to give a concentration of 0.1 mg/mL. The reaction mixtures were incubated in a water bath at 37°C for 1 h. At the end of the incubation period, 0.1 mL of 0.1 M ethylenediaminetetraacetic acid (EDTA) was added to stop the reaction (the iron–EDTA complex is unreactive in the bleomycin assay). DNA damage was assessed by adding 1 mL 1% (w/v) TBA and 1 mL 25% (v/v) HCl followed by heating in a water bath maintained at 80°C for 15 min. The chromogen formed was extracted into butan-l-ol, and the absorbance was measured at 532 nm.

Acknowledgments. The authors would like to thank Abdelbasset A. Farahat, Department of Chemistry, Georgia State University, Atlanta, Georgia, USA, for recording some of the ^{1}H NMR spectra on a Bruker 400 MHz spectrometer.

REFERENCES AND NOTES

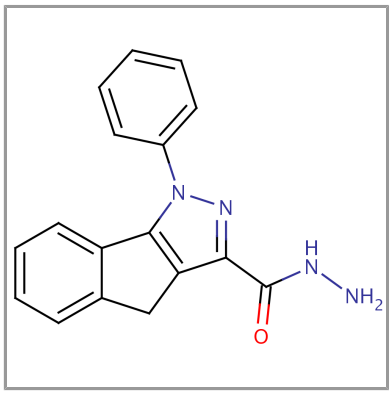
[1] Dovlatyan, V. V.; Eliazyan, K. A.; Pivazyan, V. A.; Yengoyan, A. P. Chem Heterocycl Cpds 2006, 42, 383.
[2] Smirnov, V. A.; Simerzina, L. V. Pharm Chem J 1997, 31, 589.
[3] Amr, A. E.; Sabrry, N. M.; Abdalla, M. M.; Abdel-Wahab, B. F. Eur J Med Chem 2009, 44, 725.
[4] Abdel-Wahab, B. F.; Abdel-Aziz, H. A.; Ahmed, E. M. Arch Pharm 2008, 341, 734.
[5] Abdel-Wahab, B. F.; Mohamed, S. F.; Amr, A. E.; Abdalla, M. M. Monatsh Chem 2008, 139, 1083.
[6] Chen, H.; Li, Z.; Han, Y. J Agric Food Chem 2000, 48, 5312.
[7] Rostom, S. A. F.; Shalaby, M. A.; El-Demellawy, M. A. Eur J Med Chem 2003, 38, 959.
[8] Koz'minykh, V. O. Pharm Chem J 2006, 40, 8 and references cited therein.
[9] Shaaban, M. R.; Saleh, T. S.; Mayhoub, A. S.; Farag, A. M. Eur J Med Chem 2011, 46, 3690.
[10] Mabkhot, Y. N.; Kheder, N. A.; Farag, A. M. Heterocycles 2011, 83, 609.
[11] Farag, A. M.; Dawood, K. M.; Abdel-Aziz, H. A.; Hamdy, N. A.; Fakhr, I. M. I. J Heterocycl Chem 2011, 48, 355.
[12] Farag, A. M.; Ali, K. A. K.; Eldebss, T. M. A.; Mayhoub, A. S.; Amr, A. E.; Abdel-Hafez, N. A.; Abdulla, M. M. Eur J Med Chem 2010, 45, 5887.
[13] Farag, A. M.; Mayhoub, A. S.; Eldebss, T. M. A.; Amr, A. E.; Ali, K. A. K.; Abdel-Hafez, N. A.; Abdulla, M. M. Arch Pharm 2010, 343, 384.
[14] Mabkhot, Y. N.; Kheder, N. A.; Farag, A. M. Heterocycles 2010, 81, 2369.
[15] Darwish, E. S.; Kheder, N. A.; Farag, A. M. Heterocycles 2010, 81, 2247.
[16] Abdel-Aziz, H. A.; Abdel-Wahab, B. F.; Badria, F. A. Arch Pharm 2010, 343, 152.
[17] Darweesh, A. F.; Shaaban, M. R.; Farag, A. M.; Metz, P.; Dawood, K. M. Synthesis 2010, 3163.
[18] Dawood, K. M.; Ragab, E. A.; Farag, A. M. Phosphorus, Sulfur and Silicon, Rel Elem 2010, 185, 1796.
[19] Shaaban, M. R.; Eldebss, T. M. A.; Darweesh, A. F.; Farag, A. M. J Chem Res 2010, 8.
[20] Hegazi, B.; Mohamed, H. A.; Badria, F. A.; Dawood, K. M. Chem Pharm Bull 2010, 58, 479.
[21] Farag, A. M.; Kheder, N. A.; Mabkhot, Y. N. Heterocycles 2009, 78, 1787.
[22] Kheder, N. A.; Mabkhot, Y. N.; Farag, A. M. Heterocycles 2009, 78, 937.
[23] Shaaban, M. R.; Saleh, T. S.; Farag, A. M. Heterocycles 2009, 78, 699.
[24] Shaaban, M. R.; Saleh, T. S.; Farag, A. M. Heterocycles 2009, 78, 151.
[25] Farag, A. M.; Mayhoub, A. S.; Barakat, S. E.; Bayomi, A. H. Bioorg Med Chem 2008, 16, 881.
[26] Farag, A. M.; Mayhoub, A. S.; Barakat, S. E.; Bayomi, A. H. Bioorg Med Chem 2008, 16, 4569.
[27] Shaaban, M. R.; Saleh, T. S.; Mayhoub, A. S.; Mansour, A.; Farag, A. M. Bioorg Med Chem 2008, 16, 6344.
[28] Girgis, A. S.; Mishriky, N.; Farag, A. M.; El-Eraky, W. I.; Farag, H. Eur J Med Chem 2008, 43, 1818.
[29] Kheder, N. A.; Mabkhot, Y. N.; Farag, A. M. Heterocycles 2008, 75, 2937.
[30] Kheder, N. A.; Mabkhot, Y. N.; Farag, A. M. Heterocycles 2008, 75, 887.
[31] Gutteridge, J.; Rowley, D.; Halliwell, B. Biochem J 1981, 199, 263.
[32] Siddiquia, N.; Ahujaa, P.; Ahsana, W.; Pandeyab, S. N.; Alam, M. S. J Chem Pharm Res 2009, 1, 19.
[33] Dubenko, R. G.; Gorbenko, E. F. Z Org Khim 1968, 4, 634.
[34] Biere, H.; Schroeder, E.; Ahrens, H.; Kapp, J. F. B.; Boettcher, I. Eur J Med Chem 1982, 17, 27.
[35] Stankovsky, S.; Jedlovska, E.; Spirkova, K. Coll Czech Chem Commun 1993, 58, 2211.
[36] Abramovitch, R. A.; Hey, D. H. J Chem Soc 1954, 1697.
[37] Cesu, Z. Pharmazie 1987, 42, 716.
[38] Shah, V. H.; Chauhan, N. A.; Parikh, A. R. J Ind Chem Soc 1987, 64, 678.
[39] Badria, F. A.; Ameen, M.; Akl, M. Z Naturforsch 2007, 62c, 656.
[40] El-Gazzar, A.; Gaafar, A. M.; Youssef, M. M.; Abu-Hashem, A.; Badria, F. A. Phosphorus, Sulfur, Silicon, Rel Elem 2007, 182, 2009.

2



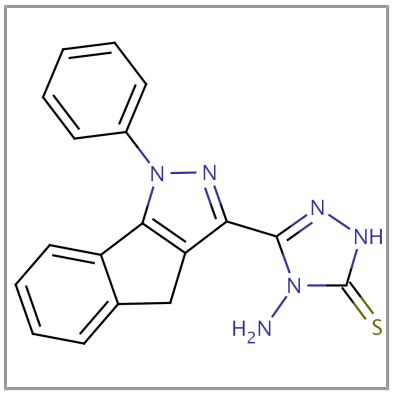
Compound Details Structure Search

1



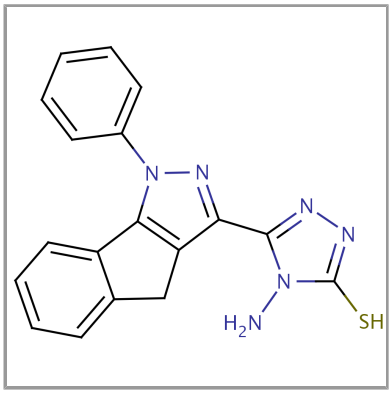
Compound Details Structure Search

3



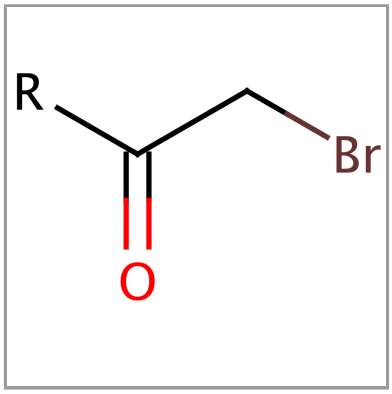
Compound Details Structure Search

3a



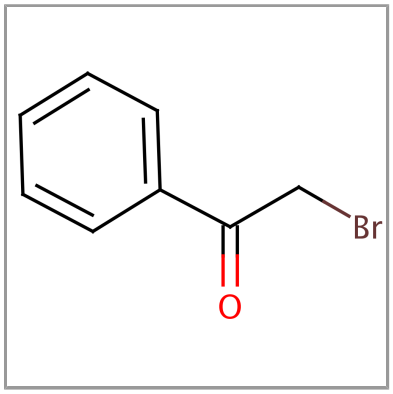
Compound Details Structure Search

4



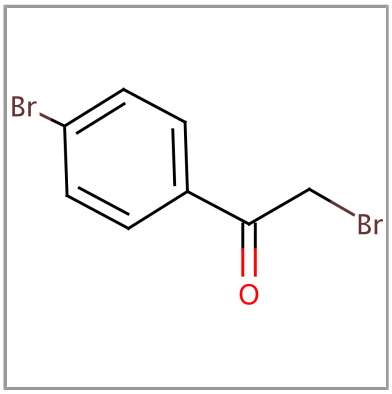
Compound Details Structure Search

4a



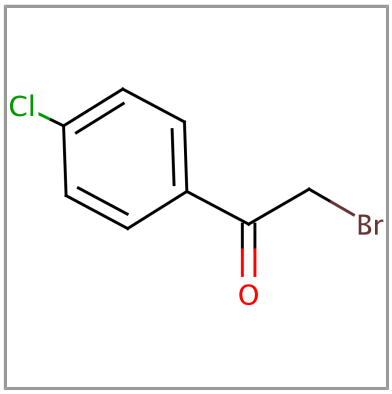
Compound Details Structure Search

4b



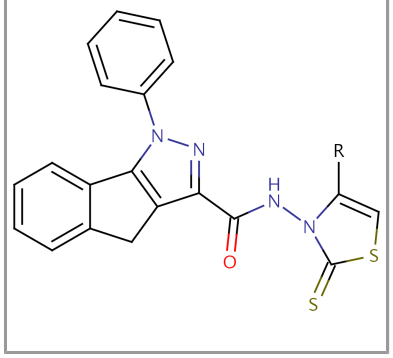
Compound Details Structure Search

4c



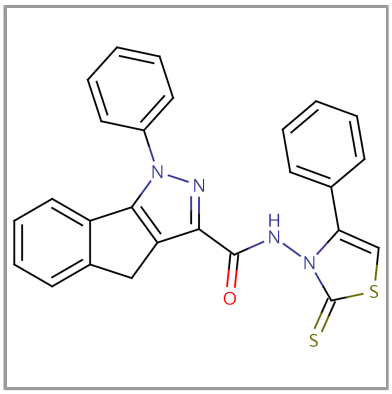
Compound Details Structure Search

5



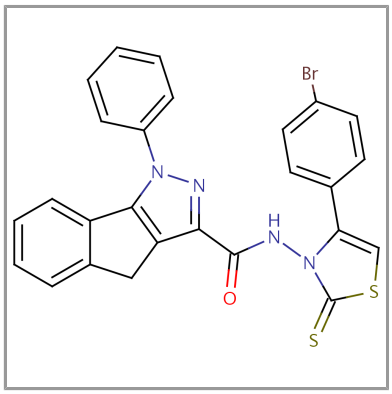
Compound Details Structure Search

5a



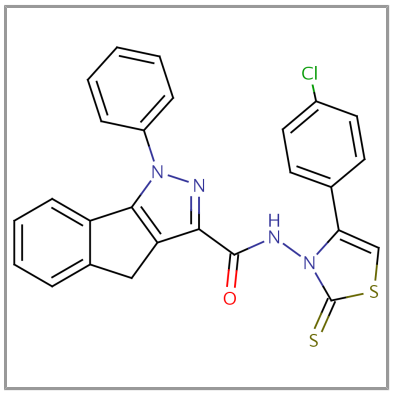
Compound Details Structure Search

5b



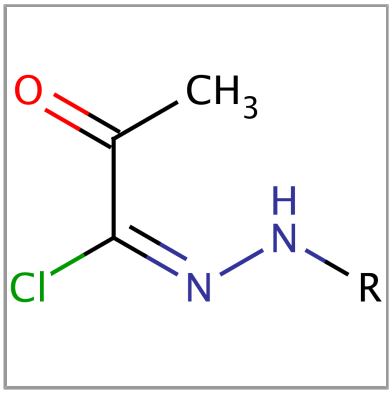
Compound Details Structure Search

5c



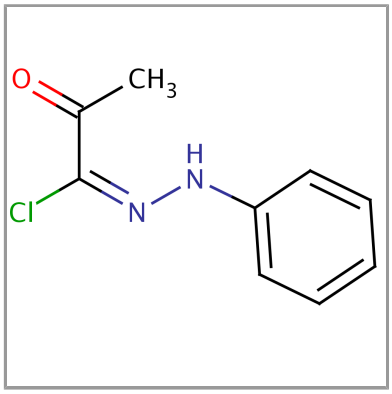
Compound Details Structure Search

6



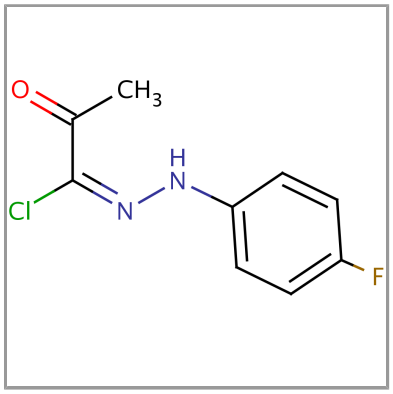
Compound Details Structure Search

6a



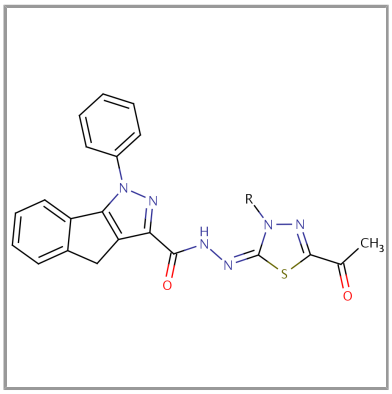
Compound Details Structure Search

6b



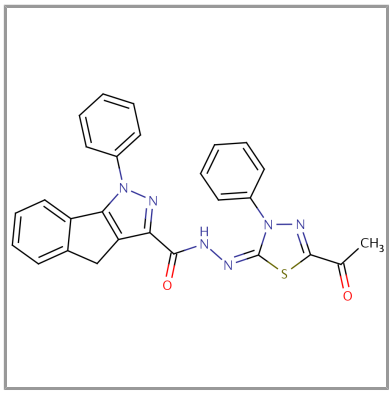
Compound Details Structure Search

7



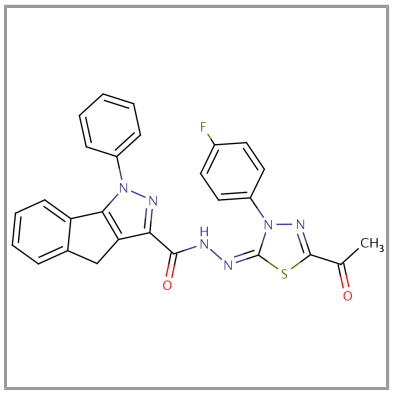
Compound Details Structure Search

7a



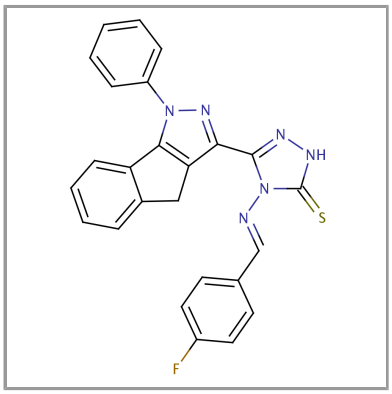
Compound Details Structure Search

7b



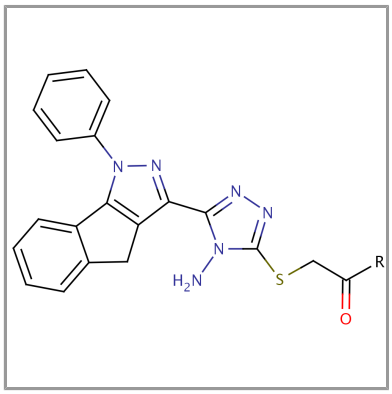
Compound Details Structure Search

8



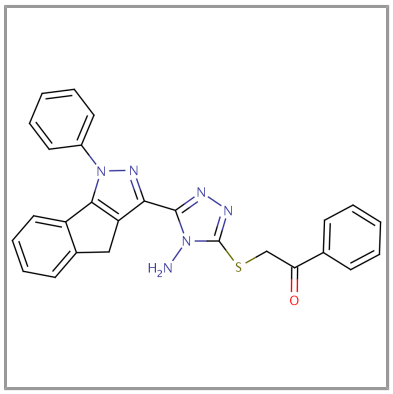
Compound Details Structure Search

9



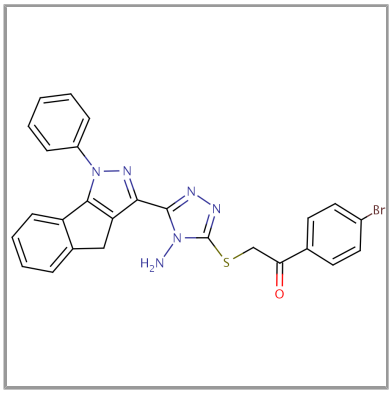
Compound Details Structure Search

9a



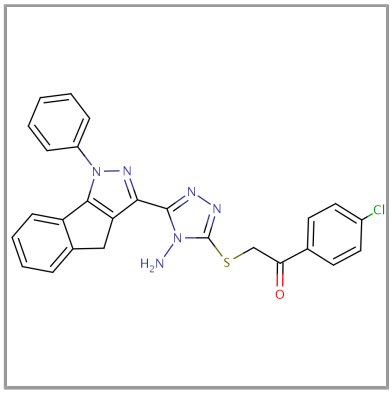
Compound Details Structure Search

9b



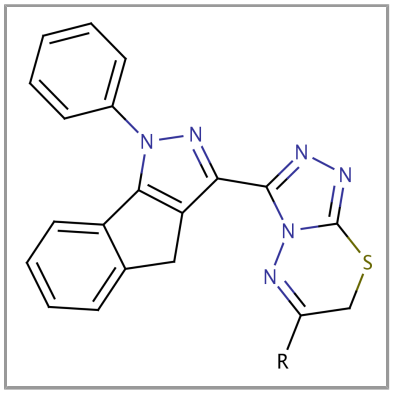
Compound Details Structure Search

9c



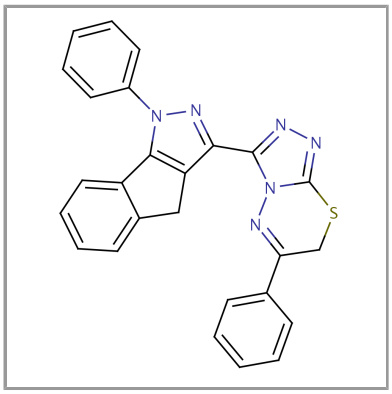
Compound Details Structure Search

10



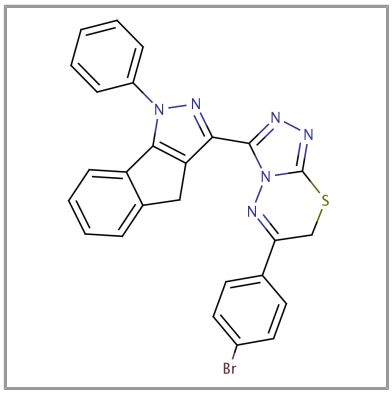
Compound Details Structure Search

10a



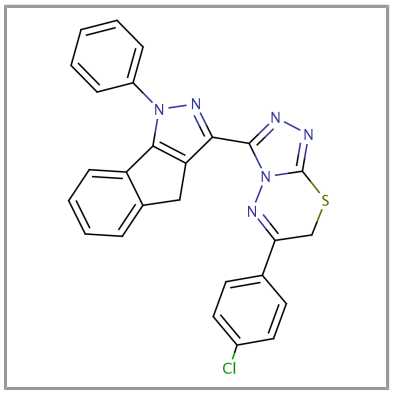
Compound Details Structure Search

10b



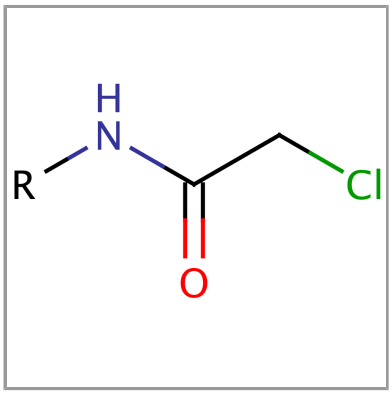
Compound Details Structure Search

10c



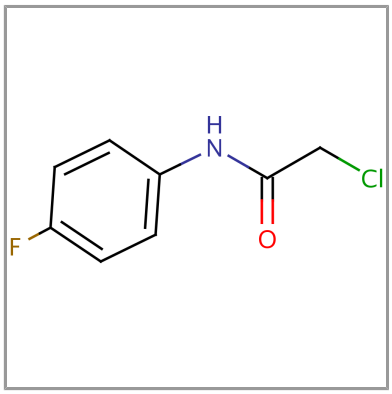
Compound Details Structure Search

11



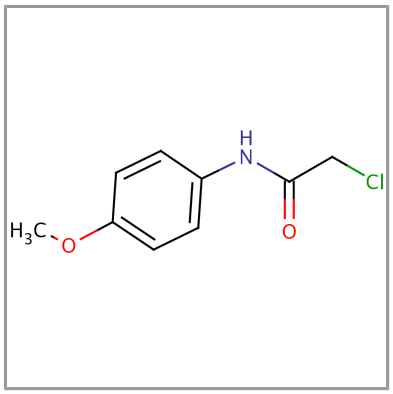
Compound Details Structure Search

11a



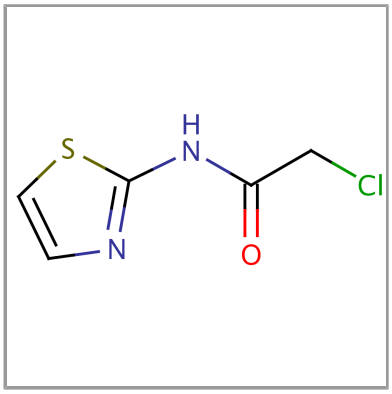
Compound Details Structure Search

11b



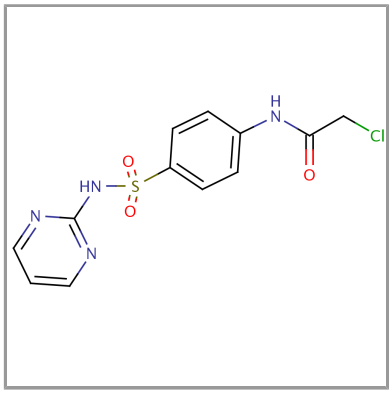
Compound Details Structure Search

11c



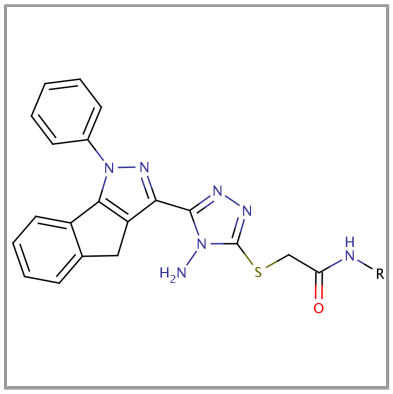
Compound Details Structure Search

11d



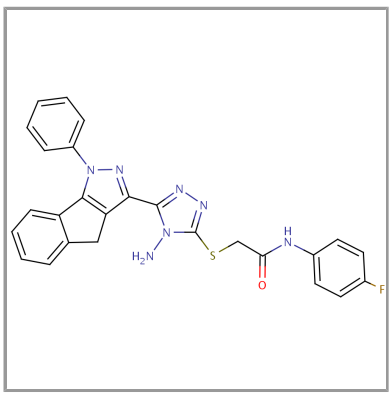
Compound Details Structure Search

12



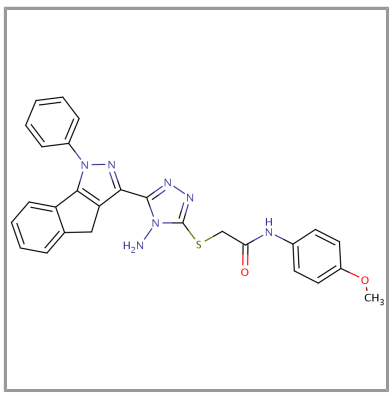
Compound Details Structure Search

12a



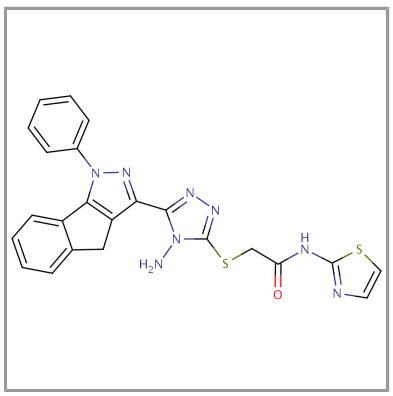
Compound Details Structure Search

12b



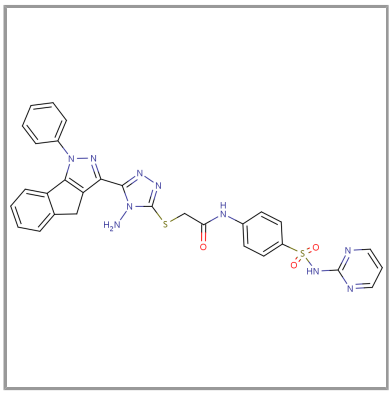
Compound Details Structure Search

12c



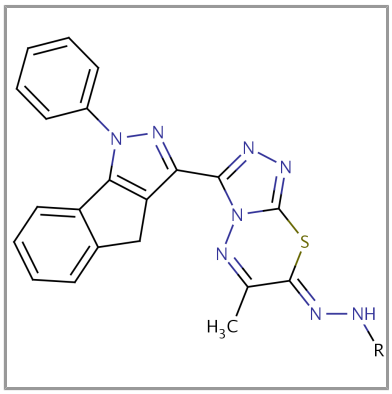
Compound Details Structure Search

12d



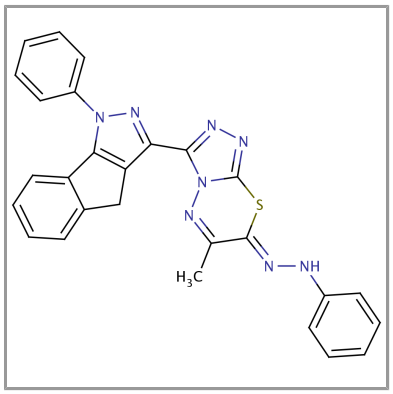
Compound Details Structure Search

13



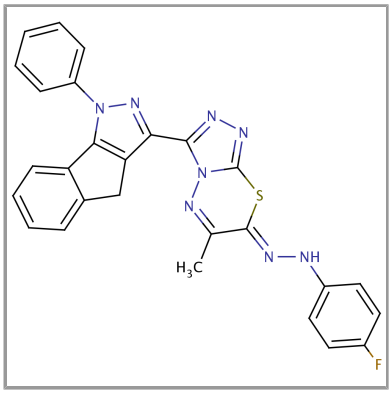
Compound Details Structure Search

13a



Compound Details Structure Search

13b



Compound Details Structure Search