Research Article

Studies on Organo-phosphorus Compounds Part II: Synthesis and biological activities of some new benzochromeno[2,3-d][1,3,2]thiazaphosphinine derivatives

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Accepted on: 17-09-2013; Finalized on: 30-11-2013.

ABSTRACT

A series of novel heterocyclic chromeno[2,3-d][1,3,2]thiazaphosphinine derivatives were synthesized in a satisfactory yield via reaction of 2-aminobenzo[f]chromene with the dimer p-methoxyphenylthiophosphine sulphide (Lawesson’s Reagent, LR). New structures were characterized by IR, NMR and MS spectra as well as elemental analyses. All the compounds were screened for their antibacterial, antifungal and toxicity using brine shrimp test (Artimia salina L.). Preliminary results indicated that some target compounds exhibited promising antibacterial and antifungal potency.

Keywords: Synthesis, organo-phosphorus compounds, antifungal, antibacterial.

INTRODUCTION

Chromenes are important class of oxygenated heterocyclic compounds. Among different types of chromene structures, 2-amino-4H-chromenes (especially 2-aminobenzochromenes) attracted our attention because of their occurrences in many natural products having spasmylytic, diuretic, anticoagulant, anticancer, anti-HIV and anti-anaphylactic activities1-9. Compounds incorporate chromenes entities have a wide range of pharmacological activities6,7 such as antidepressant, antihypertensive, anti-tubulin, antiviral, antioxidative activity8,9. Chromenes are known to activate potassium channels and inhibit phosphodiesterase IV and dihydrofolate reductases10-17.

Chromenes are also used as cosmetics, pigments18, and potential biodegradable agrochemicals19. Synthetic chromene analogues have been developed over decades, and some of them have been employed as antifungal20 and antimicrobial agents21. The current interest in 2-amino-4H-chromenes arises from their application in the treatment of human inflammatory TNFα-mediated diseases, such as rheumatoid and psoriatic arthritis and in cancer therapy (Fig. 1)22-24.

On other hand, Lawesson’s Reagent is the most effective and versatile thiation reagent for carbonyl compounds25,26. Also Lawesson’s Reagent undergoes ring closure reactions with substrates containing two functional groups27-29 forming phosphorus heterocyclic compounds, which have a wide spectrum of biological activities such as insecticidal30, antibacterial, antifungal31,32 and anticancer33.

Fig. 1: 2-Amino-4H-chromenes as privileged medicinal scaffolds

Upon continuation of our study on developing more versatile and convenient synthesis of highly functionalized heterocycles34-38 and extending to our applications on Lawesson’s Reagent (LR)39, we herein report a convenient synthetic protocol for new benzochromeno[2,3-d][1,3,2]thiazaphosphinine derivatives in a good to excellent yield.
MATERIALS AND METHODS

Chemistry

All melting points were recorded on Melt-Temp II melting point apparatus. IR spectra were measured as KBr pellets on a Nicolet 710 FT-IR spectrometer. $^1$H NMR spectra were recorded in deuterated dimethyl sulfoxide at 400 MHz on a Bruker NMR spectrometer using tetramethylsilane as an internal reference. Mass spectra were performed on a Shimadzu GCMS-QP 1000 mass spectrometer at 70 eV. The elemental analyses were carried out on an elemental analyzer 240C. All compounds were checked for their purity on TLC plates.

General procedures for the Synthesis of Compounds (3a-f), (5a-d) and (7a-d):

A mixture of compounds 2a-f, 4a-f or 6a-f (0.01 mol) and 2,4-Bis(4-methoxyphenyl)-1,3,2-dithiadiaphosphetane-2,4-disulphide (Lawesson’s Reagent, LR) in dry acetonitrile (50 mL) was refluxed until completion after 6 hours. The reaction was monitored by TLC. The reaction mixture was filtered off and the filtrate was evaporated under vacuum. The separated solid was crystallized from ethanol.

9-(4-Methoxyphenyl)-9-sulfide-8,12-dihydro-9H,11H-benzo[5,6]chromeno-[2,3-d][1,3,2]thiazaphosphinin-11-imine (3a)

Yield (70%); crystallized from EtOH; mp190°C; IR (KBr) cm$^{-1}$: $\nu$ 3300 (NH), 3040 (CH$_{aromt}$), 644 (P=5); $^1$H-NMR (400 MHz, d$_6$-DMSO): $\delta$ 8.12-7.11 (m, 15H, CH$_{aromt}$), 6.22 (s, 2H, 2NH), 4.07 (s, 3H, OCH$_3$), 3.73 (s, H, CH); MS, m/z (%): 500 (M$^+$) (12), 484 (18), 452 (1.7), 423 (8), 340 (4), 326 (22), 297 (29), 276 (79), 221(100). Anal. Calcd. C$_{32}$H$_{27}$N$_2$O$_5$P$_2$S$_5$ (500.56): C (64.79); H (4.23); N (5.60); P (6.19); S(12.81). Found: C (64.50); H (4.33); N (5.69); P (6.29); S(12.71).

12-(4-Chlorophenyl)-9-sulfide-9-(4-methoxyphenyl)-8,12-dihydro-9H,11H-benzo[5,6]chromeno-[2,3-d][1,3,2]thiazaphosphinin-11-imine (3b)

Yield (75%); crystallized from EtOH; mp 210 °C; IR (KBr) cm$^{-1}$: $\nu$ 3276 (NH), 3070 (CH$_3$), 1625 (C=O), 1423 (23), 1325 (25), 1283 (26), 1233 (27), 1177 (28), 1166 (29), 1148 (30), 1055 (31), 965 (9), 814 (10), 737 (11), 677 (12), 625 (P=5); $^1$H-NMR (400 MHz, d$_6$-DMSO): $\delta$ 7.99-6.87 (m, 14H, CH$_{aromt}$), 6.23 (s, 2H, 2NH), 3.96 (s, 3H, OCH$_3$), 3.63 (3H, OCH$_3$), 3.41 (s, 1H, CH); MS, m/z (%): 530 (M$^+$) (65), 514 (13), 484 (0.17), 481 (3), 453 (6), 422 (7), 344 (3), 327 (32), 268 (3) 221(100), 202 (5), 139 (36). Anal. Calcd. C$_{32}$H$_{27}$N$_2$O$_5$P$_2$S$_5$ (530.59): C (63.38); H (4.37); N (5.28); P (5.84); S (12.08). Found: C (63.20); H (4.29); N (5.38); P (5.90); S (12.18).

12-(4-Hydroxyphenyl)-9-sulfide-9-(4-methoxyphenyl)-8,12-dihydro-9H,11H-benzo[5,6]-chromeno[2,3-d][1,3,2]thiazaphosphinin-11-imine (3d)

Yield (68%); crystallized from EtOH; mp 187 °C; IR (KBr) cm$^{-1}$: $\nu$ 3173 (NH), 3030 (CH$_{aromt}$), 2964 (CH$_{aliph}$), 615 (P=5); $^1$H-NMR (400 MHz, d$_6$-DMSO): $\delta$ 7.78-6.45 (m, 14H, CH$_{aromt}$), 5.97 (s, 3H, 2NH, OH), 3.81 (s, 3H, OCH$_3$), 3.22 (s, 1H, CH). Anal. Calcd. C$_{32}$H$_{27}$N$_2$O$_5$P$_2$S$_5$ (516.56): C (62.78); H (4.11); N (5.42); P (6.00); S (12.41). Found: C (62.44); H (4.22); N (5.32); P (6.21); S (12.53).

12-(2-Hydroxyphenyl)-9-sulfide-9-(4-methoxyphenyl)-8,12-dihydro-9H,11H-benzo[5,6]-chromeno[2,3-d][1,3,2]thiazaphosphinin-11-imine (3e)

Yield (78%); crystallized from EtOH; mp 200 °C; IR (KBr) cm$^{-1}$: $\nu$ 3200 (NH), 3018 (CH$_{aromt}$), 2910 (CH$_{aliph}$), 646 (P=5); $^1$H-NMR (400 MHz, d$_6$-DMSO): $\delta$ 7.66-6.34 (m, 14H, CH$_{aromt}$), 5.98 (s, 3H, 2NH, OH), 4.61 (s, 3H, OCH$_3$), 3.60 (s, 1H, CH). Anal. Calcd. C$_{32}$H$_{27}$N$_2$O$_5$P$_2$S$_5$ (516.56): C (62.78); H (4.11); N (5.42); P (6.00); S (12.41). Found: C (62.44); H (4.22); N (5.32); P (6.21); S (12.53).

9-(4-Methoxyphenyl)-9,11-dithiooxo-12-phenyl-8,12-dihydro-9H,11H-benzo[5,6]-chromeno[2,3-d][1,3,2]thiazaphosphinine (3f)

Yield (76%); crystallized from EtOH; mp 225 °C; IR (KBr) cm$^{-1}$: $\nu$ 3197 (NH), 3000 (CH$_{aromt}$), 2900 (CH$_{aliph}$), 650 (P=5); $^1$H-NMR (400 MHz, d$_6$-DMSO): $\delta$ 8.00-7.42 (m, 15H, CH$_{aromt}$), 6.22 (s, 2H, 2NH), 4.87 (s, 3H, OCH$_3$), 3.67 (s, 1H, CH). MS, m/z (%): 517 (M$^+$) (5), 504 (6), 474 (1.7), 438 (2), 406 (6), 331 (4), 297 (100), 253 (7), 238 (192) (24), 118 (44). Anal. Calcd. C$_{32}$H$_{25}$N$_2$O$_5$P$_2$S$_5$ (517.55): C (62.65); H (3.89); N (2.71); P (5.98); S (18.58). Found: C (62.75); H (3.69); N (2.93); P (5.88); S (18.56).

4-Mino-2-(4-methoxyphenyl)-2-sulfide -5-phenyl-1,5-dihydro-2H,4H-chromeno[2,3-d][1,3,2]thiazaphosphine-8-ol (5a)

Yield (88%); crystallized from EtOH; mp142°C; IR (KBr) cm$^{-1}$: $\nu$ 3176 (NH), 3043 (CH$_{aromt}$), 696 (P=5); $^1$H-NMR (400 MHz, d$_6$-DMSO): $\delta$ 7.92-6.87 (m, 12H, CH$_{aromt}$), 6.32 (s, 3H, 2NH, OH), 4.66 (s, 3H, OCH$_3$), 3.67 (s, H, CH); MS, m/z (%): 466 (M$^+$) (19), 402 (22), 401 (30), 371 (21), 289 (23), 220 (21), 197 (100), 157 (27). Anal. Calcd. C$_{34}$H$_{27}$N$_2$O$_5$P$_2$S$_5$ (466.50): C (59.22); H (4.10); N (6.00); P (6.64); S(13.74). Found: C (59.42); H (4.22); N (6.22); P (6.30); S(13.54).
5-(4-Chlorophenyl)-4-imino-2-(4-methoxyphenyl)-2-sulfide-1,5-dihydro-2H,4H-chromeno-[2,3-d][1,3,2]thiazaphosphinophosphin-8-ol (5b).

Yield (85%); crystallized from EtOH; mp167°C; IR (KBr) cm⁻¹: 3199 (NH), 3027 (CH₉), 675 (P=5); ¹H-NMR (400 MHz, d₅-DMSO): δ 8.75-8.78 (m, 11H, CH₉), 6.47 (s, 3H, 2NH, OH), 4.35 (s, 3H, OCH₃), 3.88(s, H, CH). Anal. Calcld. C₉H₉NO₃P₅S₆ (250.95): C (55.15); H (3.62); Cl (7.08); N (5.59); P (6.18); S(12.80). Found: C (55.25); H (3.42); Cl (7.18); N (5.53); P (6.18); S(12.85).

4-Imino-2,5-di(4-methoxyphenyl)-2-sulfide-1,5-dihydro-2H,4H-chromeno-[2,3-d][1,3,2]thiazaphosphinophosphin-8-ol (5c).

Yield (89%); crystallized from EtOH; mp181°C; IR (KBr) cm⁻¹: 3170 (NH), 3000 (CH₉), 644 (P=5); ¹H-NMR (400 MHz, d₅-DMSO): δ 7.82-7.77 (m, 12H, CH₉), 6.55 (s, 3H, 2NH, OH), 4.66 (s, 3H, OCH₃), 4.32 (s, 3H, OCH₃), 3.81(s, H, CH). MS, m/z (%): 497 (M⁺) (12); 481 (2), 464 (0.86), 432 (0.68), 325 (0.47), 276 (4), 202 (3.8), 188 (100), 170 (44), 124 (61). Anal. Calcld. C₉H₉NO₃P₅S₆ (496.53): C (58.06); H (4.26); N (5.64); P (6.24); S(12.91). Found: C (58.26); H (4.24); N (5.44); P (6.24); S(12.93).

8-Hydroxy-2-(4-methoxyphenyl)-2,3-dithioxo-5-phenyl-1,5-dihydro-2H,4H-chromeno-[2,3-d][1,3,2]thiazaphosphinophosphin (5d).

Yield (89%); crystallized from EtOH; mp 205°C; IR (KBr) cm⁻¹: 3200 (NH), 3010 (CH₉), 608 (P=5); ¹H-NMR (400 MHz, d₅-DMSO): δ 8.12-7.43 (m, 13H, CH₉), 6.44 (s, 2H, NH, OH), 4.36 (s, 3H, OCH₃), 3.55(s, H, CH). MS, m/z (%): 483 (M⁺) (4), 451 (2), 433 (2), 370 (2), 293 (3), 221 (12), 212 (19), 198 (17), 197 (100), 115 (23). Anal. Calcld. C₉H₉NO₃P₅S₆ (483.53): C (57.13); H (3.75); N (2.90); P (6.41); S(19.89). Found: C (57.33); H (3.93); N (2.55); P (6.51); S(19.74).

2,10-Di(4-methoxyphenyl)-2,10-disulfide-5,7-diphenyl-1,5,10,11-tetrahydro-2H,4H,7H,8H-[1,3,2]thiazaphosphinophosphin[5⁺,4⁺,5⁺,6⁺]pyrano[3',2',6',7']chromeno-[2,3-d][1,3,2]thiazaphosphinophosphin-4,8-diimine (7a).

Yield (67%); crystallized from EtOH; mp 245°C; IR (KBr) cm⁻¹: 3182 (NH), 3030 (CH₉), 621 (P=5); ¹H-NMR (400 MHz, d₅-DMSO): δ 9.23 (s, 4H, 4NH), 7.92-6.13 (m, 20H, CH₉), 4.98 (s, 6H, 2OCH₃), 3.78(s, 2H, 2CH). MS, m/z (%): 823 (M⁺) (41), 822 (M⁺ - 1) (23), 807 (23), 791 (26), 761 (22), 728 (25), 698 (24), 667 (28), 590 (28), 514 (22), 364 (28), 315 (38), 285 (44), 263 (100), 235 (89), 180 (8), 158 (32). Anal. Calcld. C₉H₉NO₃P₅S₆ (822.90): C (58.38); H (3.92); N (6.81); P (7.73); S(15.58). Found: C (58.44); H (3.54); N (6.82); P (7.74); S(15.68).

5,7-di(4-chlorophenyl)-2,10-di(4-methoxyphenyl)-2,10-disulfide-1,5,10,11-tetrahydro-2H,4H,7H,8H-[1,3,2]thiazaphosphinophosphin[5⁺,4⁺,5⁺,6⁺]pyrano[3',2',6',7']chromeno-[2,3-d][1,3,2]thiazaphosphinophosphin-4,8-diimine (7b).

Yield (75%); crystallized from EtOH; mp 200°C; IR (KBr) cm⁻¹: 3210 (NH), 3030 (CH₉), 621 (P=5); ¹H-NMR (400 MHz, d₅-DMSO): δ 9.00 (s, 4H, 4NH), 8.12-7.00 (m, 18H, CH₉), 4.28 (s, 6H, 2OCH₃), 3.35(s, 2H, 2CH). MS, m/z (%): 892 (M⁺) (6), 891 (M⁺ - 1) (7), 799 (9), 731 (9), 658 (8), 512 (10), 510 (41), 437 (15), 359 (3), 297 (24), 276 (96), 233 (25), 231 (72), 187 (100), 139 (40), 77 (41). Anal. Calcld. C₉H₈ClNO₃P₅S₆ (891.79): C (53.87); H (3.39); Cl (7.97); N (6.28); P (6.95); S(14.38). Found: C (53.68); H (3.40); Cl (7.93); N (6.38); P (6.96); S(14.48).

Pharmacology

Inhibition zone was used for testing the activity of the compounds against toxigenic bacteria and dermatophytes fungii based on the method previously described by Speer and Sussmann 1982. 50 µg of the appropriated compounds were dissolved in DMSO, evaporation of the solvent and the disc put on surface inoculated medium. The dishes were incubated at 28-37°C for 48 h to 15 days for bacteria and fungi, respectively. At the end of incubation period, the diameter of no growth was measured.

RESULTS AND DISCUSSION

Chemistry
The IR spectra of compounds 3a-e showed the absence of the absorption bands corresponding to amino and cyano groups while appearing characteristic bands at average 3200, 3100, 650 cm⁻¹ were corresponding to NH and P=S. ¹H-NMR showed the appearance of new signals between δ 6.23-5.97 ppm and 4.87 – 3.81 ppm are characteristic of 2NH protons and three aliphatic protons (OCH₃), respectively. ¹H-NMR of compound 3f showed the absence of the signals corresponding to ester group of the starting material. Mass spectra of compounds 3a, 3c and 3f showed the molecular peak ions at 500 (12%), 530 (65%) and 517 (5%), respectively.

In a similar condition, the chromene derivatives 4a-d were allowed to react individually with Lawesson's Reagent to furnish chromeno[2,3-d][1,3,2]thiazaphosphinin-8-ol derivatives 5a-d (Scheme 2).

The reaction pathway of compound 5a was assumed to proceed via the nucleophilic attack of the amino group on LR followed by addition of SH to the cyano group (Scheme 3).

The mechanism of compound 5d was proposed to proceed via a nucleophilic attack on LR followed by ring closure and elimination of ethanol molecule which subsequent by thiation to produce 8-hydroxy-2-(4-methoxyphenyl)-2,4-dithiooxo-5-phenyl-1,5-dihydro-2H, 4H-chromeno[2,3-d][1,3,2]thiazaphosphinine 5d (Scheme 4).

The structure of products 5a-d was determined by elemental analysis and spectral data (see Experimental section).

Prompted by the aforementioned results, we have also investigated the reactivity of pyrano[3,2-g]chromene towards Lawesson's Reagent. Thus, the reaction of compounds 6a-d with (LR) in 1:2 molar ratio gave the corresponding bisthiazaphosphininino[5''4''5,6'']pyrano [3',2':6,7]chromene 7a-d in high yield (Scheme 5).

The structures of the latter products were deduced from their elemental analyses and spectral data. The ¹H-NMR spectrum of compound 7a, for example, revealed the absence of the signal corresponding to amino group of the starting material and the appearance of new signals characteristic of protons corresponding to methoxy group. Mass spectra of compounds 7a, 7b and 7d showed the molecular peak ions at 823 (41%), 892 (6%), 856 (7%), respectively.
Scheme 2

Scheme 3

Scheme 4
**Table 1:** Toxicity (brine shrimp larvae) test and activity of benzochromeno[2,3-d][1,3,2]thiazaphosphinine and its derivatives against bacterial and fungal strains, respectively.

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<th>Antibacteria activity</th>
<th>Antifungal activity</th>
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**Pharmacology**

**Toxicity (Brine Shrimp Test)**

The isolated compounds were tested for toxicity using brine shrimp test (Artimia salina L); all screened compounds had high toxicity to the brine shrimp larvae.

**Antibacterial Test**

In vitro antimicrobial activity of the tested compounds summarized in Table 1 revealed the following: compounds 3a-e and 5a, 5b, 5d showed moderate activity 11-20 mm against the two types of bacteria Bacillus cereus, Staphylococcus albus (Gram positive) and Escherichia coli, Psedomonas aureginosa (Gram negative), while compounds 7a-d were proved to be highly active 20-30 mm on the same types of bacteria.

**Antifungal Test**

All the synthesized products were evaluated in vitro for their antifungal activity (dermatophytes fungi) and...
revealed that compound 7d displayed highest activity against Trichophyton mentagrophytes and Trichophyton verrucosum. Compounds 5a, 5b, 5d, 7a, 7b and 7c have moderate activity against two fungal strains, while compounds 3a-e showed weekly activity.

CONCLUSION

Lawesson’s Reagent is employed to generate new phosphorous heterocyclic compounds based chromene scaffold structures. Thus, compounds benzochromen[2,3-d][1,3,2]thiazaphosphinines 3a-f, chromen[2,3-d][1,3,2]thiazaphosphin-8-ol 5a-d and bisthiazaphosphinin[5,4;5′,6′]pyrano[3′,2′:6,7]chromene 7a-d were synthesized in a satisfactory yield. Most of such compounds exhibited a good inhibitory effect against various microbial strains and observed evidently that all compounds bearing thiazaphosphinine moiety are displayed active. So the synthesized compounds must carry out for using in medical treatments.

Acknowledgment: Authors are grateful to Manchester Metropolitan University and Sohag University for supporting and facilitating this study.

REFERENCES